

# **Three Year Summary Age and Growth Report**

**For**

**Plains minnow**  
**Western silvery minnow**  
**Brassy minnow**  
*(Hybognathus spp.)*

## **Pallid Sturgeon Population Assessment Project and Associated Fish Community Monitoring for the Missouri River**



**Prepared for the U.S. Army Corps of Engineers – Northwest Division**  
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## Executive Summary

Pallid sturgeon *Scaphirhynchus albus* are native throughout the Missouri River and the middle and lower Mississippi River. Due to human influences, population levels of this species have greatly declined over the last century. To study this species in-depth, the U.S. Army Corps of Engineers (COE) developed the Pallid Sturgeon Population Assessment Program (PSPAP).

To meet the objectives of the PSPAP, eight species of fish were collected for age and growth analysis as a representative group of native Missouri River fishes. Age-growth information is important to fisheries management because this data can be used to answer many questions and problems that exist within a fishery. Length-at-age information can be used to show trends, either positive or negative, of the condition of a species. When a management strategy is implemented, this information can be used to determine the effectiveness of the plan.

These selected Missouri River fishes were processed by the following PSPAP agencies: Sand Shiner-*Notropis stramineus*, Sauger-Sander *canadensis*, Plains Minnow, Brassy Minnow and Western Silvery Minnow-*Hybognathus* spp. (Missouri Department of Conservation), Sicklefins Chub-*Macrhybopsis meeki*, Speckled Chub-*Macrhybopsis aestivalis*, and Sturgeon Chub-*Macrhybopsis gelida*, (U.S. Fish and Wildlife Service-Columbia Fisheries Resource Office), Shovelnose Sturgeon-*Scaphirhynchus platyrhynchus*, (Nebraska Game and Parks Commission) and Blue Sucker-*Cycleptus elongatus* (South Dakota Game, Fish and Parks).

Age structures were collected from *Hybognathus* spp. during fish community seasons from August-September in 2004, July-October in 2005, and July-October in 2006. *Hybognathus* spp. were collected using otter trawls, push trawls, bag seines, mini-fyke nets, and fishing poles. During 2004, 2005 and 2006, 1,389 *Hybognathus* spp. were captured from all segments combined, and age structures were collected on 206 of these fish. Mean back calculated length at last annulus for the upper universe (segments 1-4) was 60mm at age 1 and 85mm at age 2. Mean back calculated length at last annulus for the lower universe (segments 7-14) was 52mm at age 1 and 72mm at age 2.

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## Introduction

Pallid sturgeon *Scaphirhynchus albus* are native throughout the Missouri River and the middle and lower Mississippi River. Due to human influences, population levels of this species have greatly declined over the last century. Contributions to losses include reduced water quality, habitat loss, barriers to migration and over-fishing. As a result Pallid Sturgeon were listed as endangered by the U.S. Fish & Wildlife Service in 1990 (Drobish 2007b).

The Pallid Sturgeon Recovery Plan (USFWS 1993) identified six priority pallid sturgeon recovery management areas (RPMAs), four of which lie within the Missouri River. Further, this document provided an outline that proposed to: 1) protect and restore pallid sturgeon populations, individuals, and their habitats; 2) conduct research necessary for survival and recovery of pallid sturgeon; 3) develop and implement a pallid sturgeon captive propagation program, and; 4) coordinate and implement conservation and recovery of sturgeon species (Drobish 2007b).

In 2000, the U. S. Fish and Wildlife Service (USFWS) issued the U. S. Army Corps of Engineers (COE) the Biological Opinion on the Operation of the Missouri River Main System Reservoir system Operation and Maintenance of the Missouri River Bank Stabilization and Navigation Project and Operation of the Kansas River Reservoir System (Bi-Op). This document recommended that the flow regime of the Missouri River mimic a more natural hydrograph, an increase in propagation and population augmentation efforts, and the development of a pallid sturgeon population assessment program (PSPAP). As the federal entity responsible for water management within the Missouri and Kansas River systems, the COE has an obligation under the Endangered Species Act to conserve the pallid sturgeon. To comply with the Bi-Op, the COE has proposed to operate Gavins Point Dam in a manner to create a more natural hydrograph, has funded hatchery improvements and expansions, has funded the PSPAP, and facilitated the development of the Pallid Sturgeon Population Assessment Team (Drobish 2007b).

The initial stocking of pallid sturgeon in 1994 consisted of approximately 7,000 fish from the 1992 year class that were stocked into RPMAs 4 (Missouri River below Gavins Point Dam) and 5 (middle Mississippi River). Subsequent stockings in 1997, 1998, 2000,

and 2002 through 2005 in all six RPMAs have resulted in nearly 172,000 pallid sturgeon being stocked into the Missouri and Mississippi river systems (Drobish 2007b).

Implementation of the PSPAP began in 2001 when the USFWS-Columbia Fishery Resource Office (USFWS-CFRO) began monitoring under PSPAP guidelines and Nebraska Game and Parks Commission (NGPC) conducted an evaluation of benthic trawls. The COE hired a fishery biologist to coordinate the PSPAP in 2002 and the USFWS-CFRO and NGPC continued monitoring in segments 9, 13, and 14 in the lower Missouri River. Standardized sampling above Gavins Point Dam (segments 5 and 6) occurred for the first time in 2003 by the USFWS-Great Plains Fish and Wildlife Management Assistance Office. During 2004, monitoring continued in segments 5, 6, 8, 9, 13, and 14, and an independent science review was conducted to determine the ability of the PSPAP to address its objectives. Beginning with the 2005 fish community season, the Team added the USFWS-Missouri River Fish and Wildlife Management Assistance Office (segment 4), the South Dakota Department of Game Fish and Parks (segment 7), and the Missouri Department of Conservation (segments 10 and 11). In 2006, the team added the Montana Department of Fish, Wildlife, and Parks field crew to complete implementation of the PSPAP from segment 1 through 14 (Drobish 2007b).

The objectives of the PSPAP are as follows: 1) document annual results and long-term trends in pallid sturgeon population abundance and geographic distribution throughout the Missouri River System; 2) document annual results and long-term trends of habitat use of wild pallid sturgeon and hatchery stocked pallid sturgeon by season and life stage; 3) document population structure and dynamics of pallid sturgeon in the Missouri River System; 4) evaluate annual results and long-term trends in native target species population abundance and geographic distribution throughout the Missouri River system; 5) document annual results and long-term trends of habitat usage of the native target species by season and life stage; and 6) document annual results and long-term trends of all non-target species population abundance and geographic distribution throughout the Missouri River system, where sample size is greater than fifty individuals (Drobish 2007b).

To meet objective 5 of the PSPAP, age-growth and relative weight information was collected on a representative group of native Missouri River fishes. These target species were chosen based on possible prey and habitat relationships of pallid sturgeon and those listed as Missouri River species of concern (Berry and Young 2001).

Age-growth and relative weight information is important to fisheries management. These data can be used to answer many questions and problems that exist within a fishery. Length at age information can be used to show trends, either positive or negative, of the condition of a species. When a management strategy is implemented, this information can be used to determine the effectiveness of the plan (DeVries and Frie 1996).

The selected Missouri River fishes were processed by the following PSPAP agencies: Sand Shiner-*Notropis stramineus*, Sauger-*Sander canadensis*, Plains Minnow, Brassy Minnow and Western silvery Minnow-*Hybognathus spp.* (Missouri Department of Conservation), Sicklefin Chub-*Macrhybopsis meeki*, Speckled Chub-*Macrhybopsis aestivalis*, and Sturgeon Chub-*Macrhybopsis gelida*, (USFWS-Columbia Fisheries Resource Office), Shovelnose Sturgeon-*Scaphirhynchus platyrhynchus*, (Nebraska Game and Parks) and Blue Sucker-*Cyprinus elongatus* (South Dakota Game, Fish and Parks).

## Study Area

The Missouri River was divided into segments for the PSPAP based on changes in physical attributes of the river (e.g., tributary influence, geology, turbidity, degrading or aggrading stream bed, etc.) (Figure 1). These segments were numbered 1 through 14 in a downstream direction and included all riverine portions of the Missouri River from Fort Peck Dam to the confluence (Table 1). Segments were also divided into an upper and lower sampling universe based on longitudinal difference as well as the length of the fish's growing season. Segments 1 through 4 make up the "upper sampling universe"; it is characterized by a meandering, often braided channel that lacks navigation structures. Segments 1 through 4 lie in RPMA 2 and includes the 203.5 river miles from Fort Peck Dam downstream to the headwaters of Lake Sakakawea, North Dakota.

Segments 5 through 14 make up the "lower sampling universe"; the lower sampling universe is characterized by having been highly engineered from its original state. Segments 5 and 6, lie in RPMA 3, and consist of 55 river miles from Fort Randall Dam, South Dakota, downstream to the headwaters of Lewis and Clark Lake, Nebraska-South Dakota. Segment 7 extends from Gavins Point Dam downstream 61 miles to Lower Ponca Bend, Nebraska-South Dakota, and is the only segment below Gavins Point Dam that is not channelized.

Segments 8 through 14 of the lower universe include the entire channelized portion (750 miles) of the Missouri River that extends from Lower Ponca Bend to the confluence with the Mississippi River. The Kansas River, from the Johnson County Weir (Kansas) to the mouth (15.4 miles), was given its own segment designation (segment 11) because this tributary was addressed by the 2000 Bi-Op as a high priority management area for pallid sturgeon (Caton et al. 2007).

## **Methods**

All sampling was conducted in accordance with the guidelines established by the Pallid Sturgeon Assessment Team as outlined in the Missouri River Standard Operating Procedures for Sampling and Data Collection (Drobish 2007a) and Pallid Sturgeon Population Assessment Program, (Drobish 2007b). Two distinct sampling seasons were established to assess sturgeon species and associated fish community. The sturgeon sampling season begins 1 November, or when water temperature drops below 12.8°C, and continues until 30 June. Gear types used during this season include gill nets, trammel nets, otter trawls, and hoop nets. Fish community season runs from 1 July and continues through 31 October. Gear types used during the fish community season include trammel nets, benthic otter trawls, hoop nets, mini-fyke nets, push trawls, beam trawls, and bag seines.

### Sampling Gears

***Otter Trawl*** - Two different benthic otter trawls (OT) were used to sample a variety of river habitats with water greater than 1.2 m in depth: OT16 and OT01. The OT16 and OT01 had a 4.9 m (16 ft.) head-rope and a 0.9 m mouth height. The OT16 was 7.6 m long with size 110 mesh around the cod end. The OT01 was 7.2 m long with 4 mm mesh around the cod end. The towing warp consisted of 13 mm low-stretch nylon line with a 13.7-m bridle. Otter trawls were deployed from the stern or the bow of a jet boat while traveling in a downstream direction. A buoy and line were attached to the cod end of the trawls for retrieval if a snag was encountered. Standard trawl hauls ranged from a minimum distance of 75 m to a maximum distance of 300 m. Standard paired wooden otter doors (762 mm [30 in.] x 381 mm [15 in.]) were used on all otter trawls.

***Push Trawl*** - Push trawls (POT2) were used to sample water between 0.25 m and 2.0 m off the bow of a jet boat while traveling in a downstream direction. They were deployed by mechanical means using forward facing outriggers of sufficient length to allow the net to fish ahead of the point where the boat breaks the water. Rope was then let out to accommodate for varying depths. Standard trawl hauls ranged from a minimum distance of 15 m to a maximum distance of 150 m. All push trawls were designed with a 2.4 m (8 ft.) headrope, 0.6 m mouth height, and an overall length of 1.8 m. Paired wooden doors were 762 mm (30 in.) x 381 mm (15 in.).

***Bag Seine*** - Bag seines (BS) were used to sample water less than 1.2 m using three seine haul configurations: quarter arc, half arc, and rectangular. Seining with any method could be conducted in an upstream or downstream direction. Standard seine hauls covered a minimum of 50 m<sup>2</sup> of river bottom. Bag seines were constructed from 6.4 mm ace mesh, were 9.1 m (30 ft.) in length and 1.8 m (6 ft.) in depth. Bag dimensions were 1.8 m x 1.8 m x 1.8 m. Seines were attached at each end to 1.8 m x 51 mm brails (Kennedy et al. 2005).

***Mini-fyke Net*** - Mini-fyke nets (MF) were set in shallow, slack water areas with the lead extending perpendicular to the river bank or sand bar. In areas with moderate flow, nets were positioned at a slight downstream angle with weights attached to the upstream side of the cab to prevent the net from overturning. The perpendicular distance measured from the midpoint of the cab to the bank was recorded. Nets were generally set in the afternoon and left overnight with a maximum soak time of 24 hours. Mini-fyke nets were constructed from 3-mm ace or delta mesh with two rectangular frames 1.2 m wide and 0.6 m high to form the cab. The body of the net was constructed with two 0.6 m steel hoops, with a single, 51-mm throat. The lead was 4.5-m in length and 0.6 m high (Kennedy et al. 2005).

***Fishing Pole*** - Fishing poles (FP) were used to sample a variety of habitats from both the bank and an anchored boat. Spinning rods were used to fish the bottom using a hook baited with a worm.

### Data Collection and Analysis

Plains Minnow/Western Silvery Minnow (*Hybognathus spp.*) aging structures were collected from ten fish for each 10 millimeter length group for each segment, species, and year. Aging structures from fish of all size were collected during the fish community season (July 1 – October 31) in all segments, and there was no cut-off for the maximum length of fish from which structures were collected for individuals. The entire fish was preserved in the field, properly identified in the lab, and sent to the Missouri Department of Conservation in Chillicothe, MO for age and growth assessment.

Each preserved *Hybognathus* sample was received in a separate vial labeled with field office, segment, date of capture, unique id, and fish number. Scales were removed from each fish between the lateral line and dorsal fin. Scales were cleaned either by hand with a microbrush or in an ultrasonic cleaner as outlined in the Missouri River SOP (Drobish 2007a). Cleaned scales were then placed between two glass slides and labeled with identifying information.

Images from prepared structures were digitally captured using a Paxcam 3 digital microscope camera mounted on an Olympus SZ61TR stereo microscope using Sigmascan 5 software. Structures were recorded on the monitor at a magnification of 106.2X. Captured images were named with all pertinent information in the title, including field office, river segment, unique ID, collection season year, fish identification number, and structure type. They were then saved to the appropriate folder according to collection season year, species, and segment number.

Two readers independently read each scale, recording annuli number and location. Ages were compared, and any difference in age was discussed until a concert agreement was reached. Sigmascan was then used to measure the cumulative distance (in pixels), first from the focus to each annuli, then from the focus to the outer edge. Annuli in scales were determined to be the outermost border of closely spaced circuli before growth resumed in the spring causing circuli to be spaced farther apart and more defined (DeVries and Frie 1996).

Literature found on the family Cyprinidae suggests that *Hybognathus spp.* form their annuli in late March to June (Fuchs 1967; Summerfelt and Minckley 1969). Thus, collecting *Hybognathus spp.* from July through October eliminates the concern of collecting age structures when annuli are forming.

The Fraser-Lee method was used in determining back calculated length at age for *Hybognathus spp.* (DeVries and Frie 1996). For this model a species specific y-intercept is required. An extensive literature search was conducted to find a published y-intercept for this species, producing no results. Therefore, we determined the y-intercept using a representative random sample of 50 fish (5 per 10 mm length group). The prepared *Hybognathus spp.* scales were viewed under an Olympus SZ61 microscope equipped with a Paxcam 3 digital microscope camera with Sigma Scan 5.0 imaging software. The scale radius (i.e., the center of the focus to the scale edge) was measured to the nearest hundredth of a millimeter. A linear regression plotting total length of the fish at capture (Y) against the scale radius (X), resulted in a line of  $y = 38.896x + 13.85$ , with an  $r^2$  value of 0.90 (Appendix A). Regression results indicated that scale formation (when  $x = 0$ ) occurred when fish length (y) was estimated at 13.85mm.

All aging data were entered into a Microsoft Excel spreadsheet. Statistical analysis was done using SAS 9.1 and Excel. Data were processed using a parametric ANOVA, Tukey multiple comparison test, linear regression, and a t-test. Heincke's method (Isely and Grabowski 2007) was used to calculate annual mortality. SigmaPlot 9.0 was used to produce figures.



Table 1. Description of each segment of the Missouri River with its corresponding river miles.

Segment Number	Segment Description	Upper River Mile	Lower River Mile	Length (mi)
1	Fort Peck Dam to the confluence of the Milk River	1771.5	1760.0	11.5
2	Confluence of the Milk River to Wolf Point	1760.0	1701.0	59.0
3	Wolf Point to the confluence of the Yellowstone River	1701.0	1582.0	119.0
4	Confluence of the Yellowstone River to the headwaters of Lake Sakakawea	1582.0	1568.0	14.0
5	Fort Randall Dam to the confluence of the Niobrara River	880.0	845.0	35.0
6	Confluence of the Niobrara River to the headwaters of Lewis and Clark Lake	845.0	825.0	20.0
7	Gavins Point Dam to Lower Ponca Bend	811.0	750.0	61.0
8	Lower Ponca Bend to the confluence of the Platte River	750.0	595.0	155.0
9	Confluence of the Platte River to the confluence of the Kansas River	595.0	367.5	227.5
10	Confluence of the Kansas River to the confluence of the Grand River	367.5	250.0	117.5
11	Lower Kansas River, Johnson County Weir to mouth	15.4	0	15.4
13	Confluence of the Grand River to the confluence of the Osage River	250.0	130.0	120.0
14	Confluence of the Osage River to the confluence with the Mississippi River	130.0	0.0	130.0

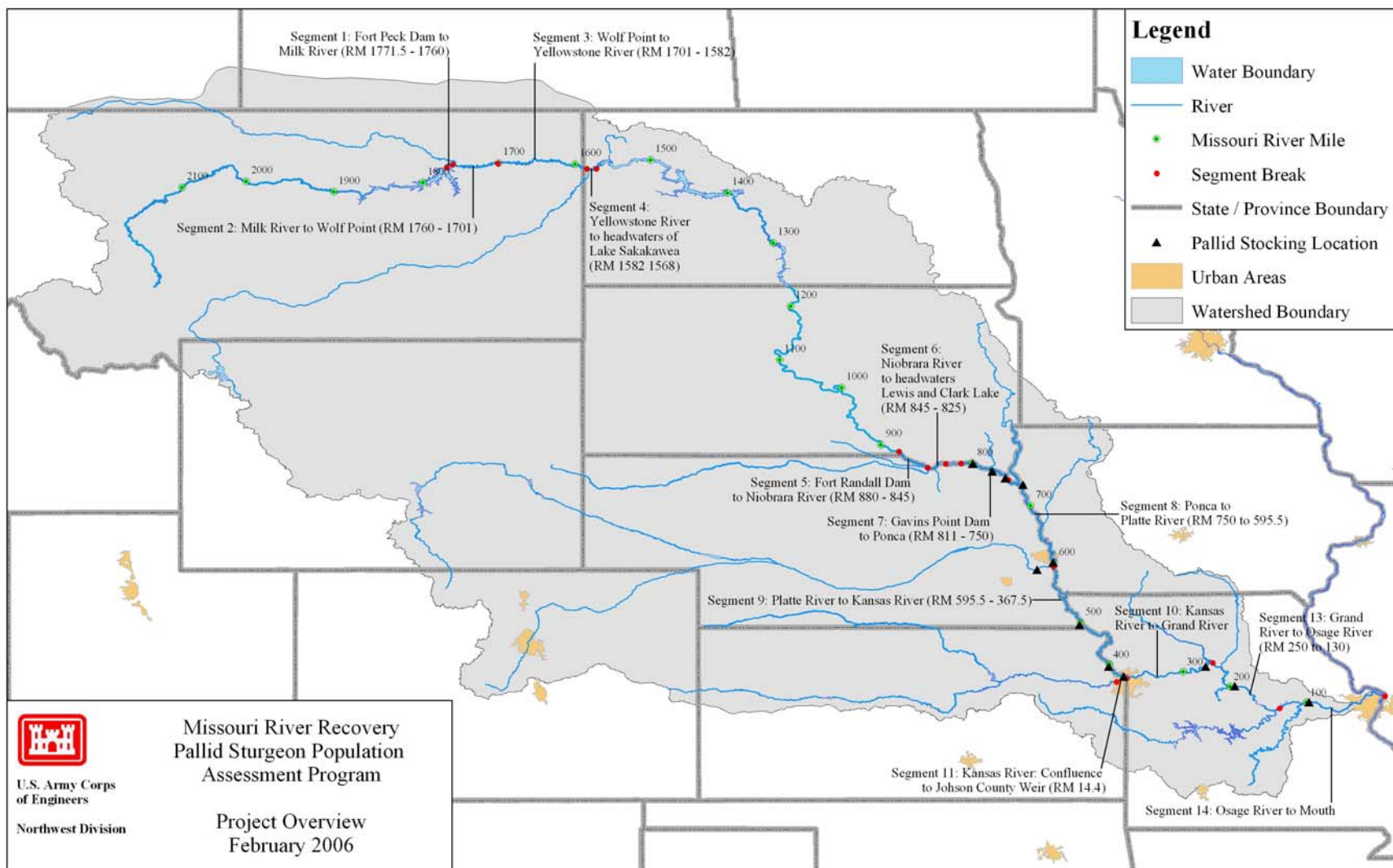


Figure 1. Map of the Missouri River basin.

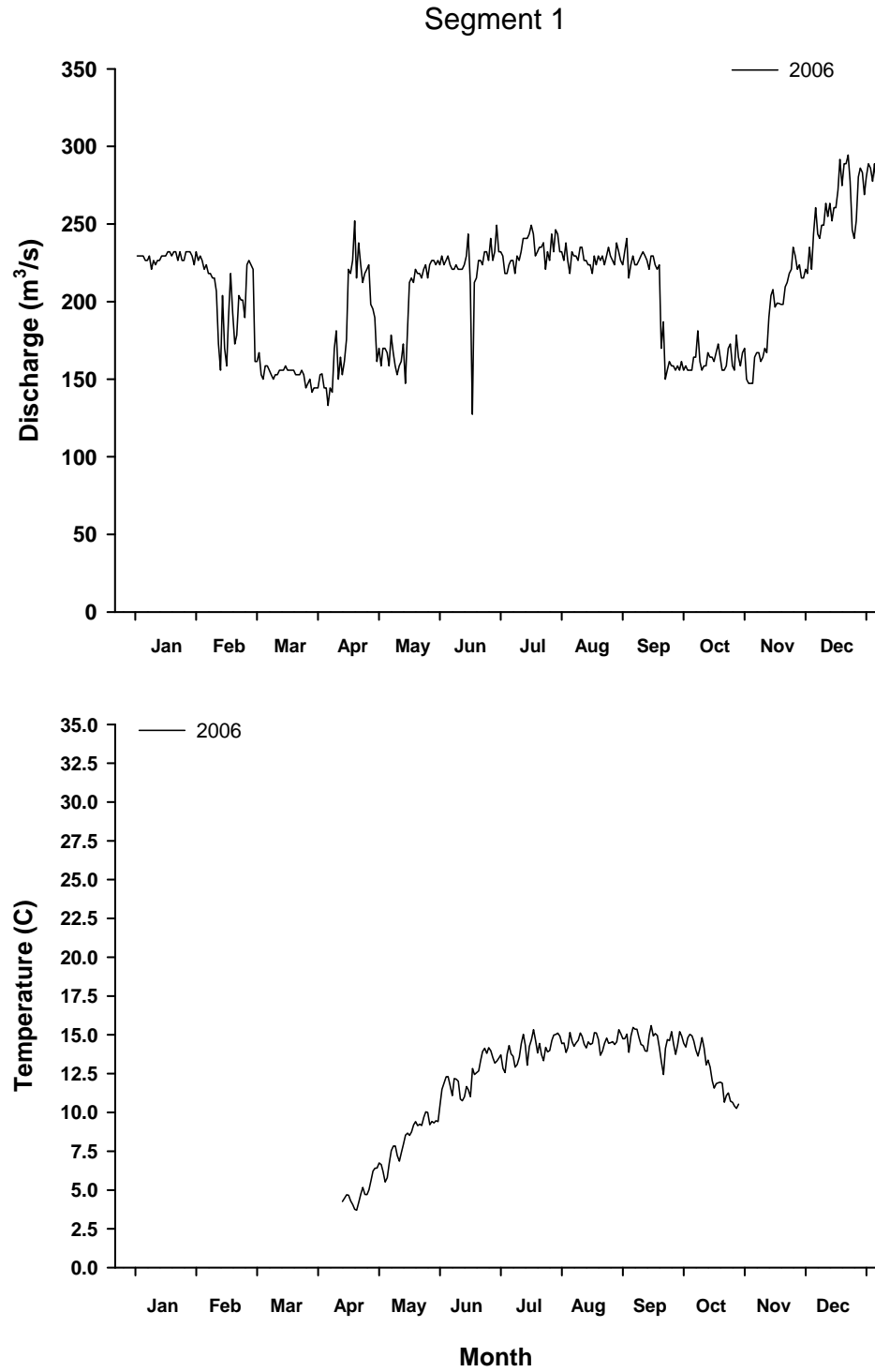


Figure 2. Mean daily discharge and mean daily water temperature for segment 1 of the Missouri River during 2006

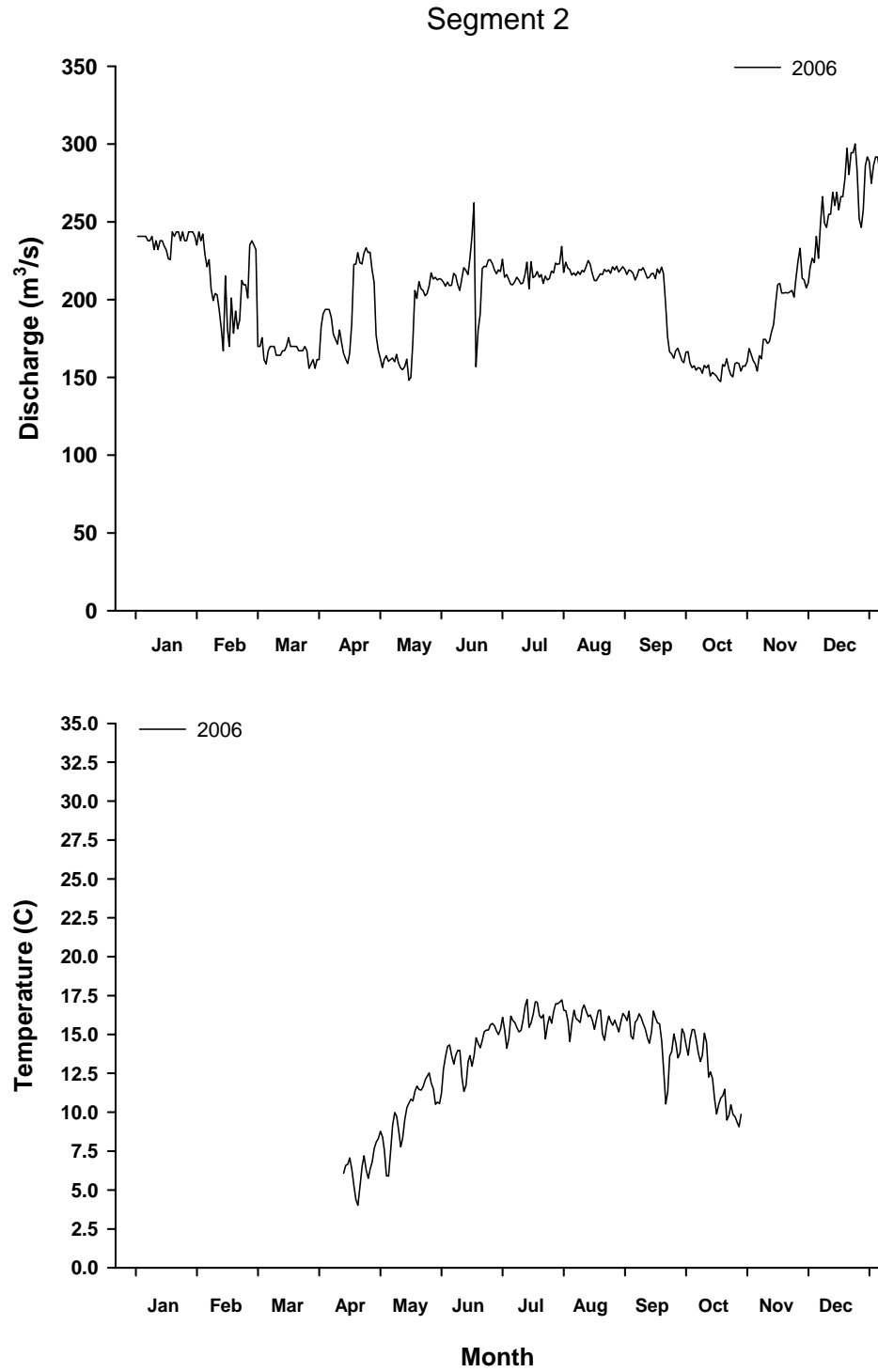


Figure 3. Mean daily discharge and mean daily water temperature for segment 2 of the Missouri River during 2006

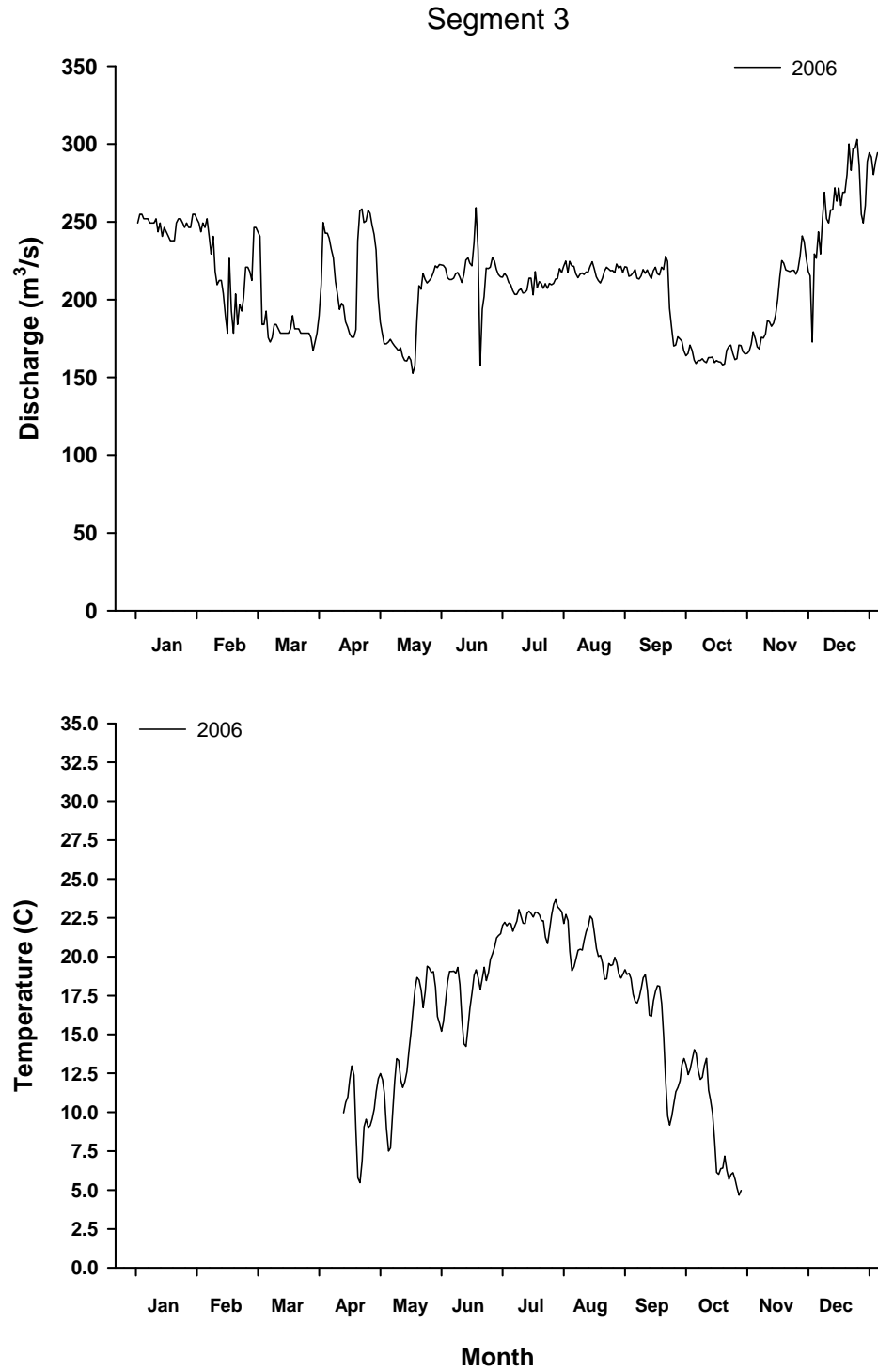


Figure 4. Mean daily discharge and mean daily water temperature for segment 3 of the Missouri River during 2006

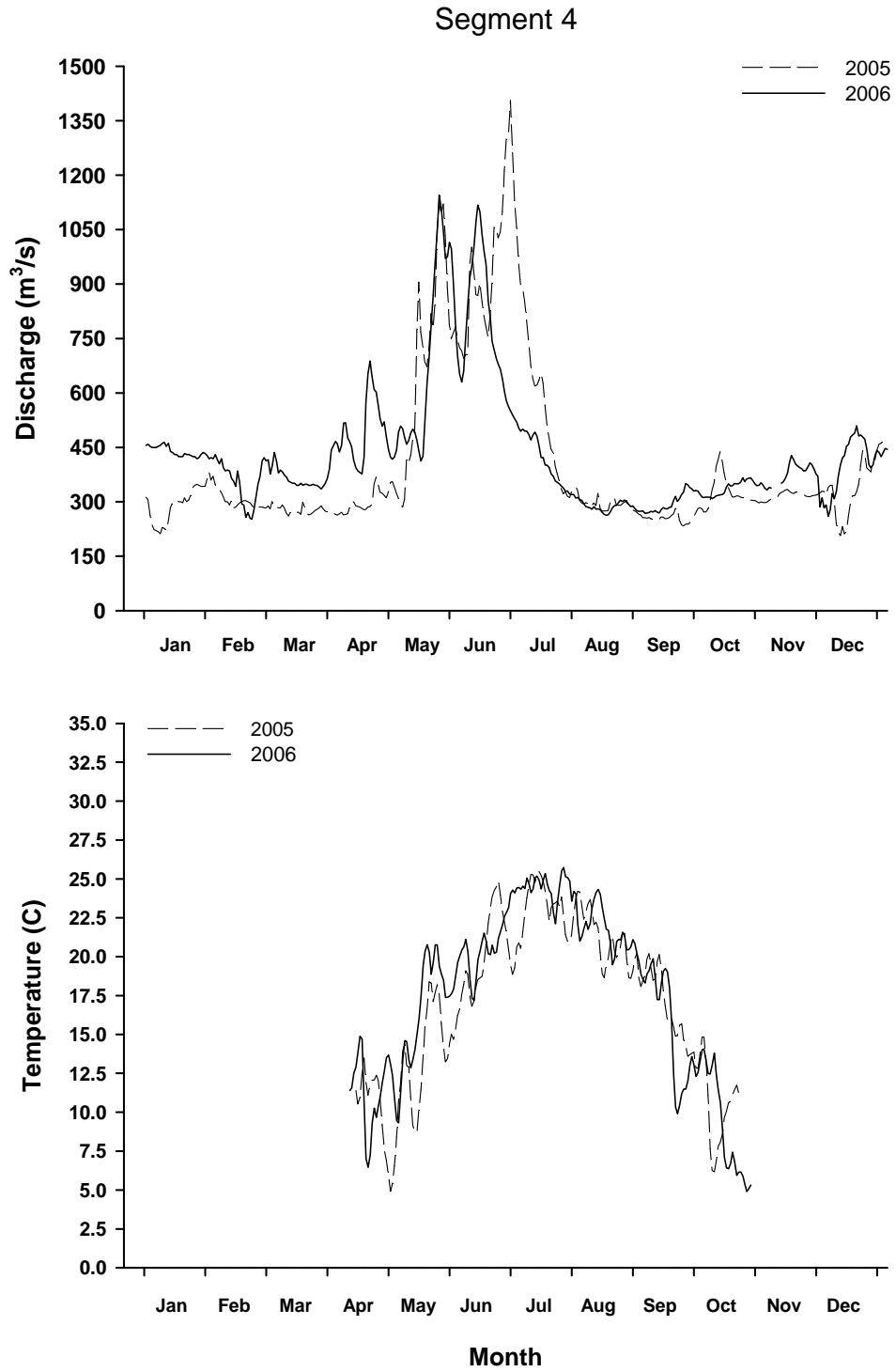


Figure 5. Mean daily discharge and mean daily water temperature for segment 4 of the Missouri River during 2005 and 2006.

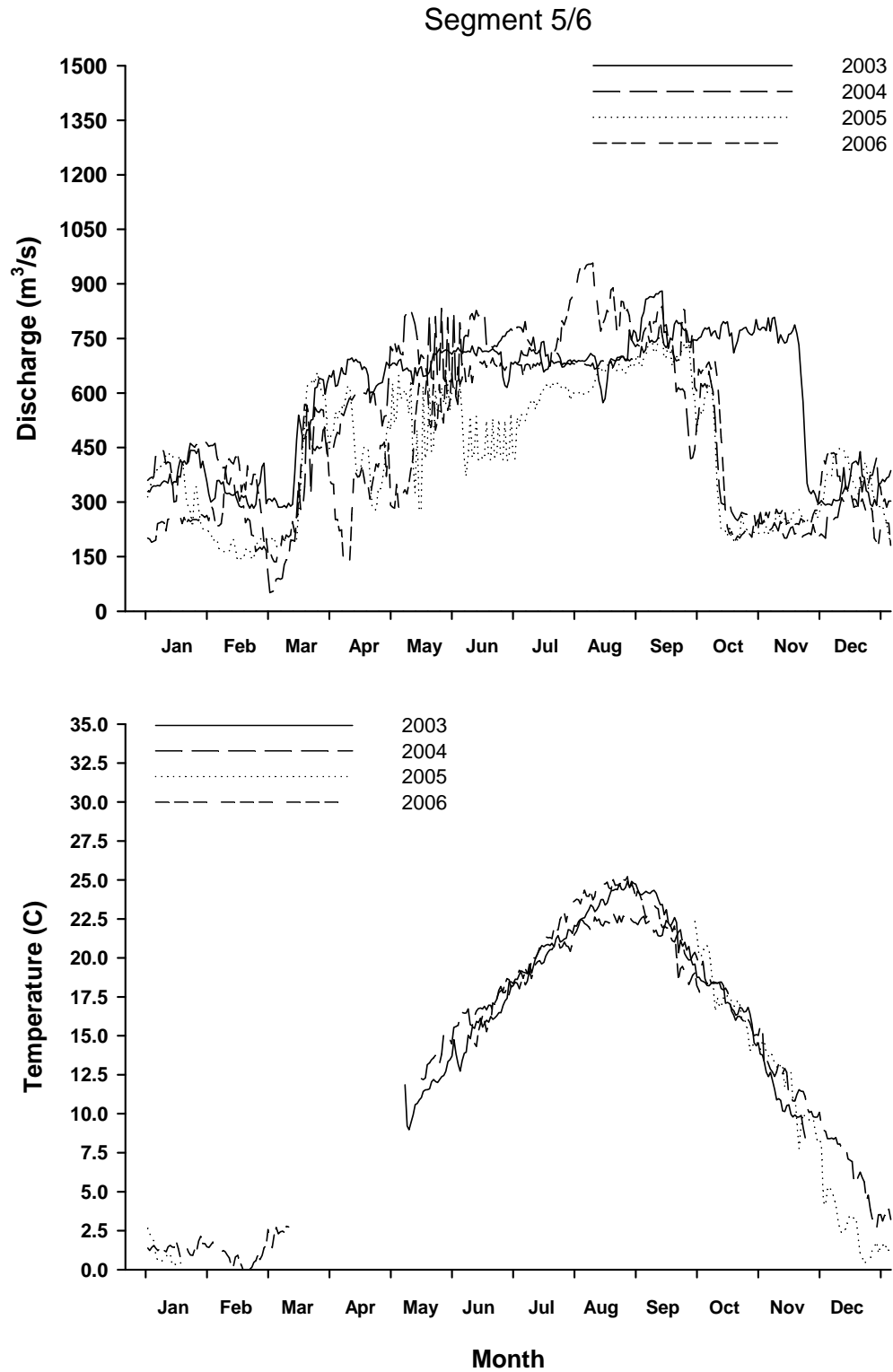


Figure 6. Mean daily discharge and mean daily water temperature for segment 5/6 of the Missouri River during 2003 through 2006.

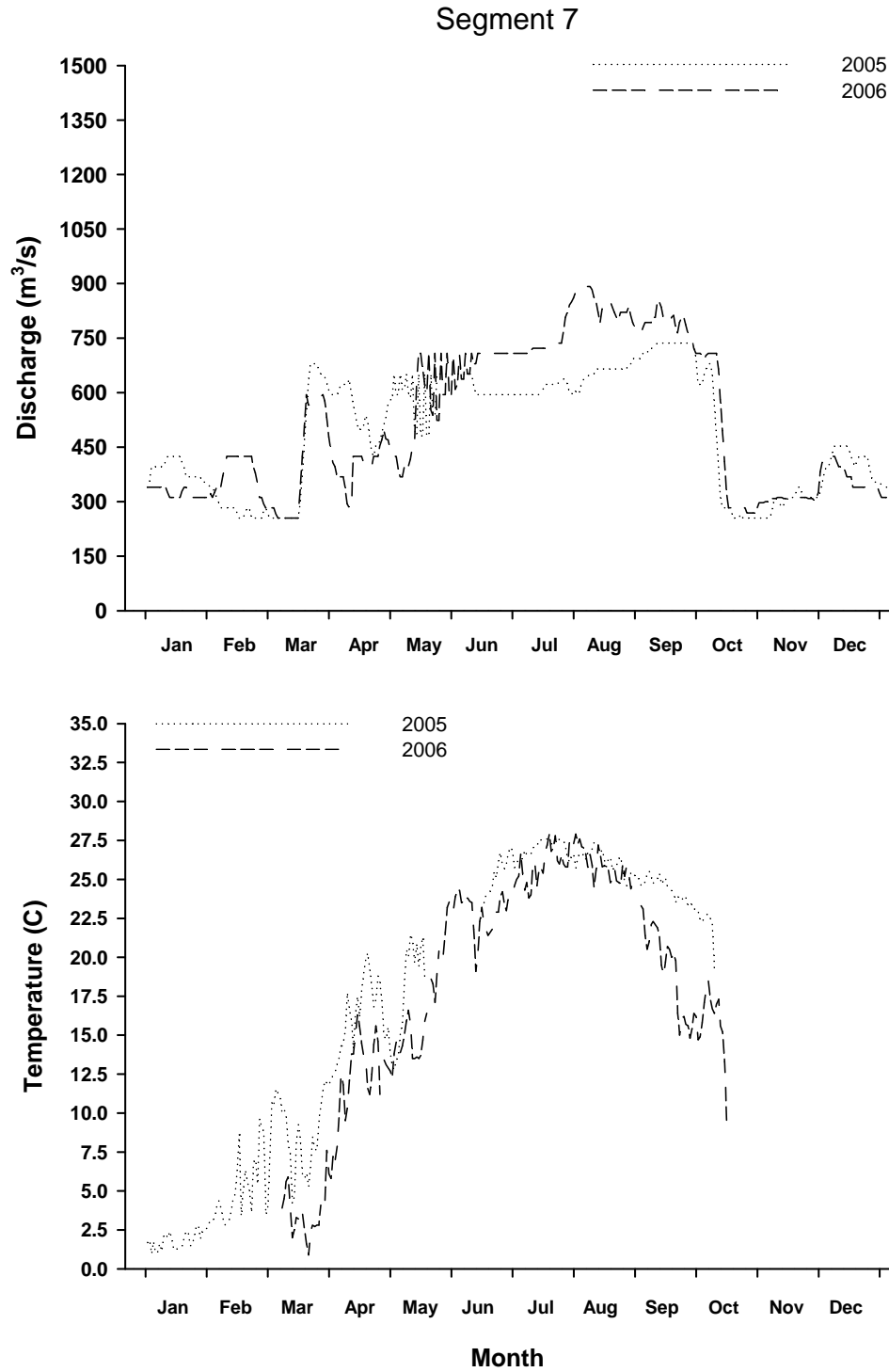


Figure 7. Mean daily discharge and mean daily water temperature for segment 7 of the Missouri River during 2005 and 2006.



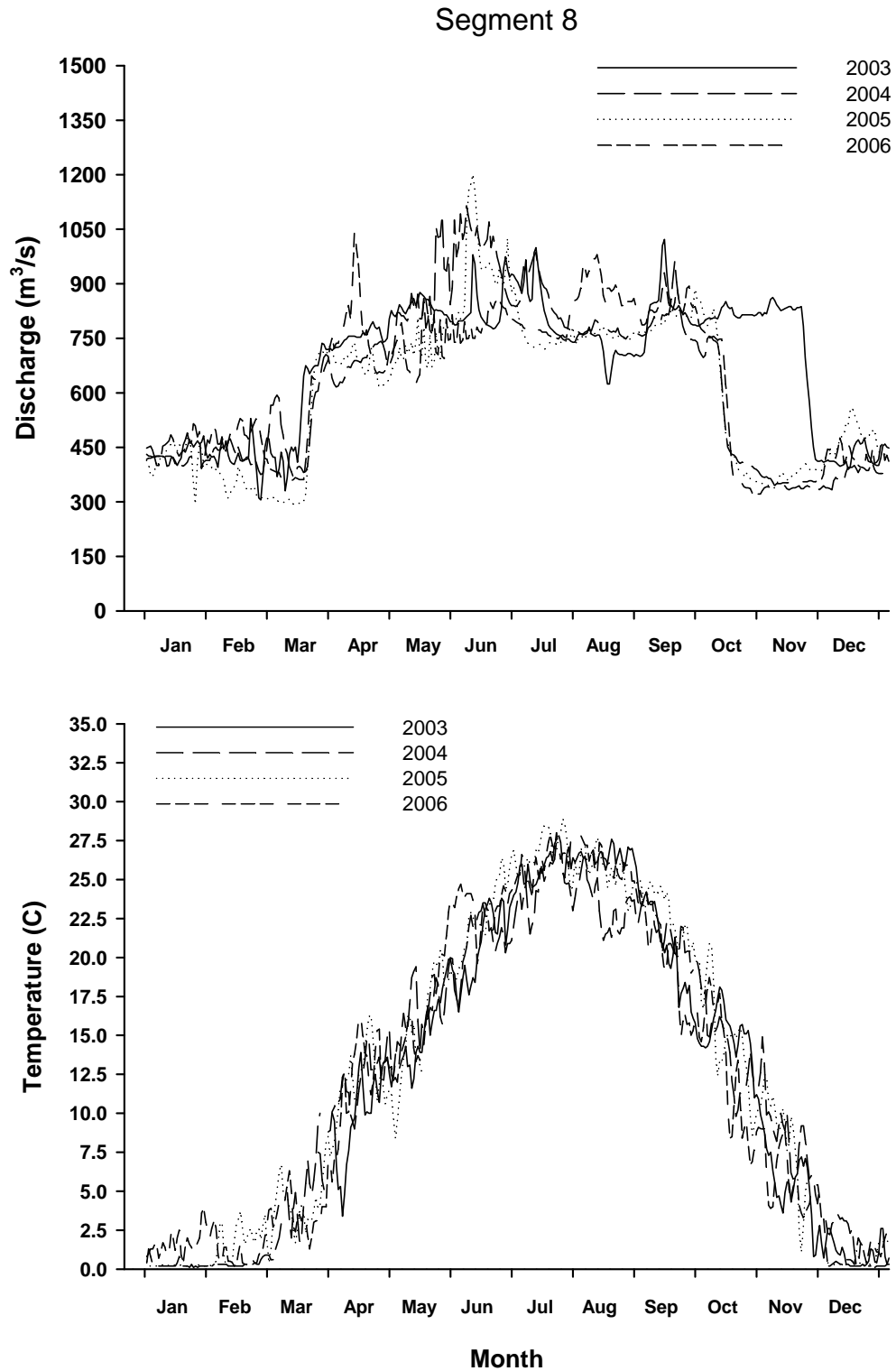


Figure 8. Mean daily discharge and mean daily water temperature for segment 8 of the Missouri River during 2003 through 2006.

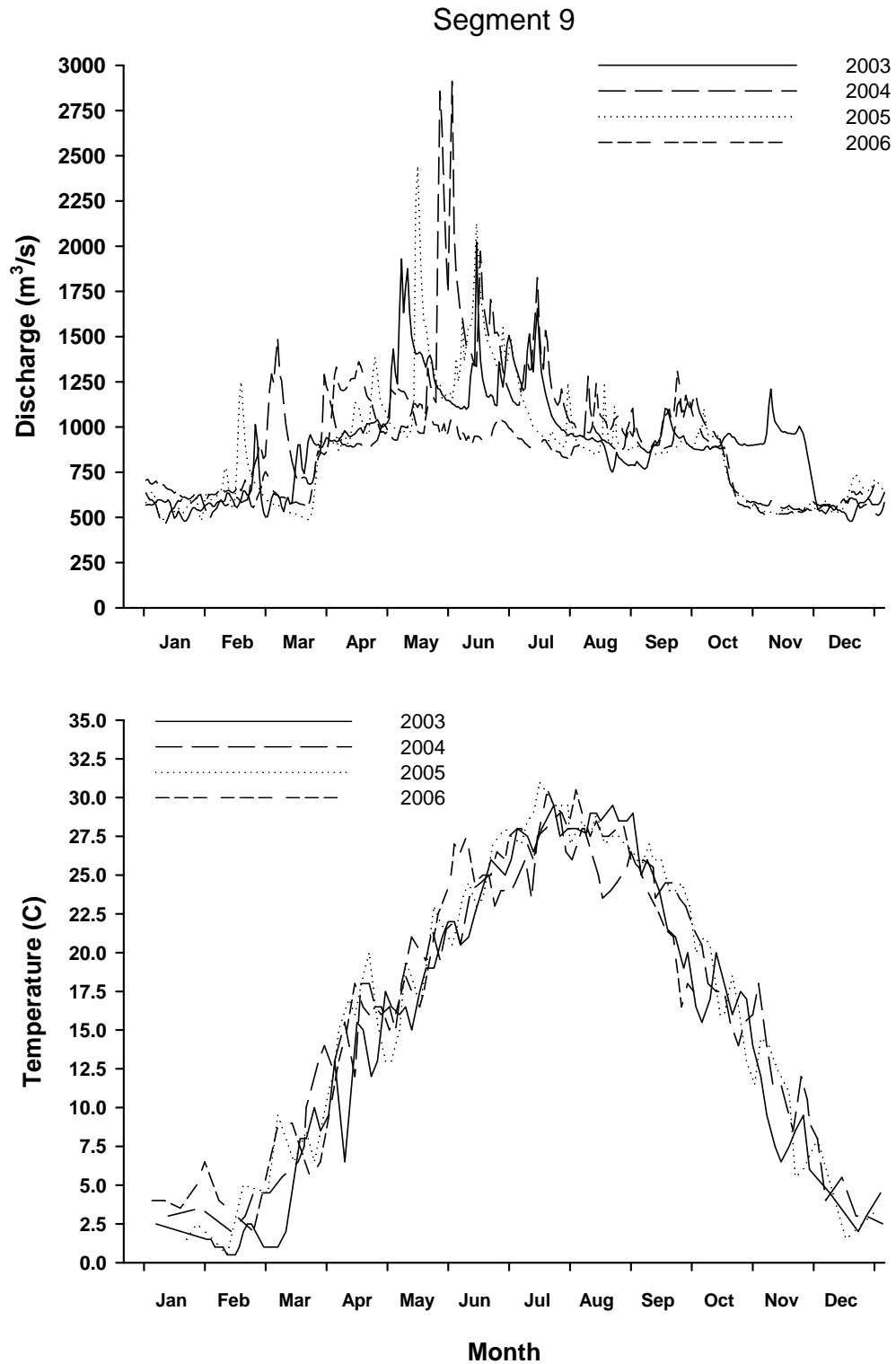


Figure 9. Mean daily discharge and mean daily water temperature for segment 9 of the Missouri River during 2003 through 2006.

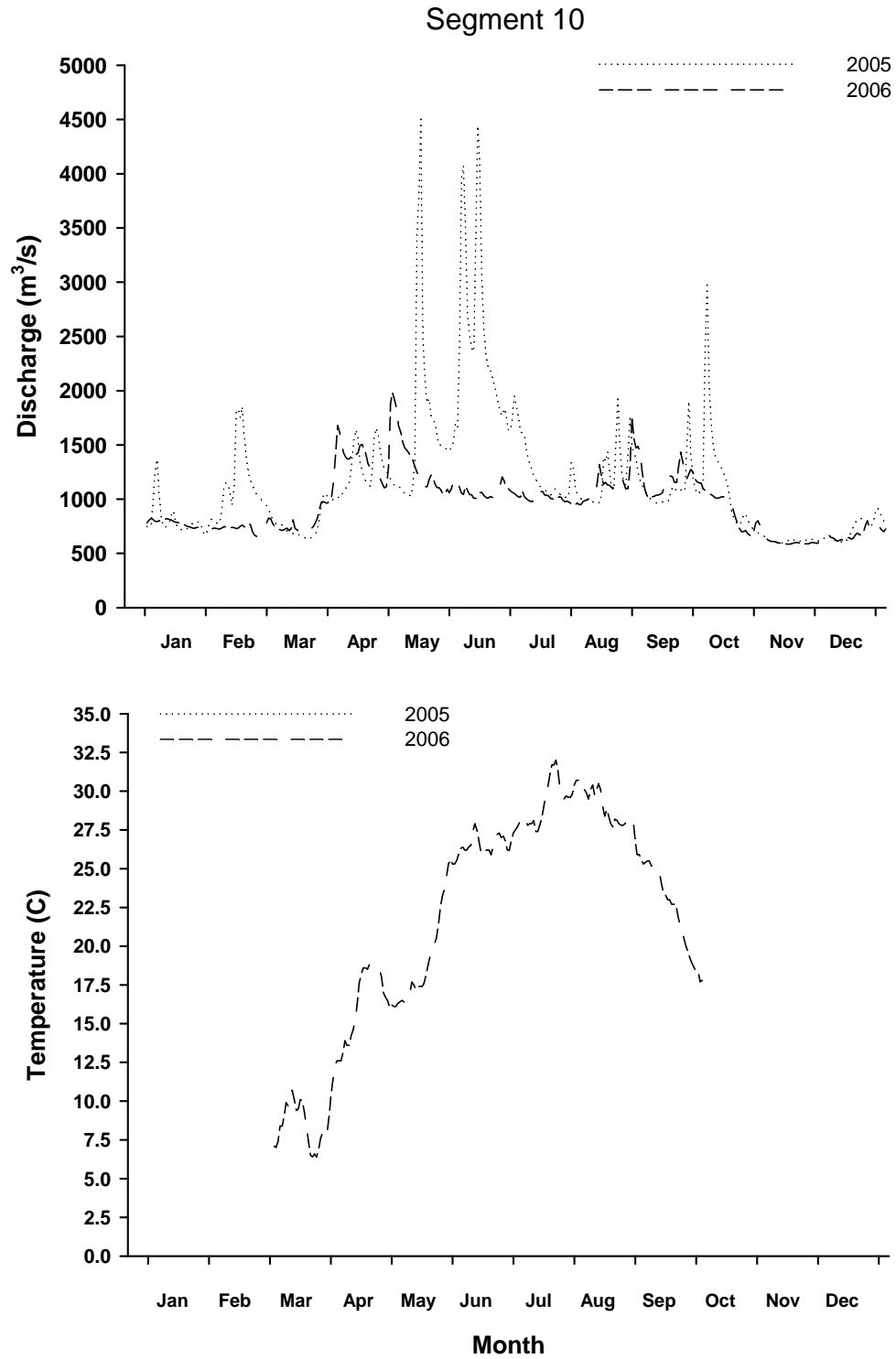


Figure 10. Mean daily discharge and mean daily water temperature for segment 10 of the Missouri River during 2005 and 2006.

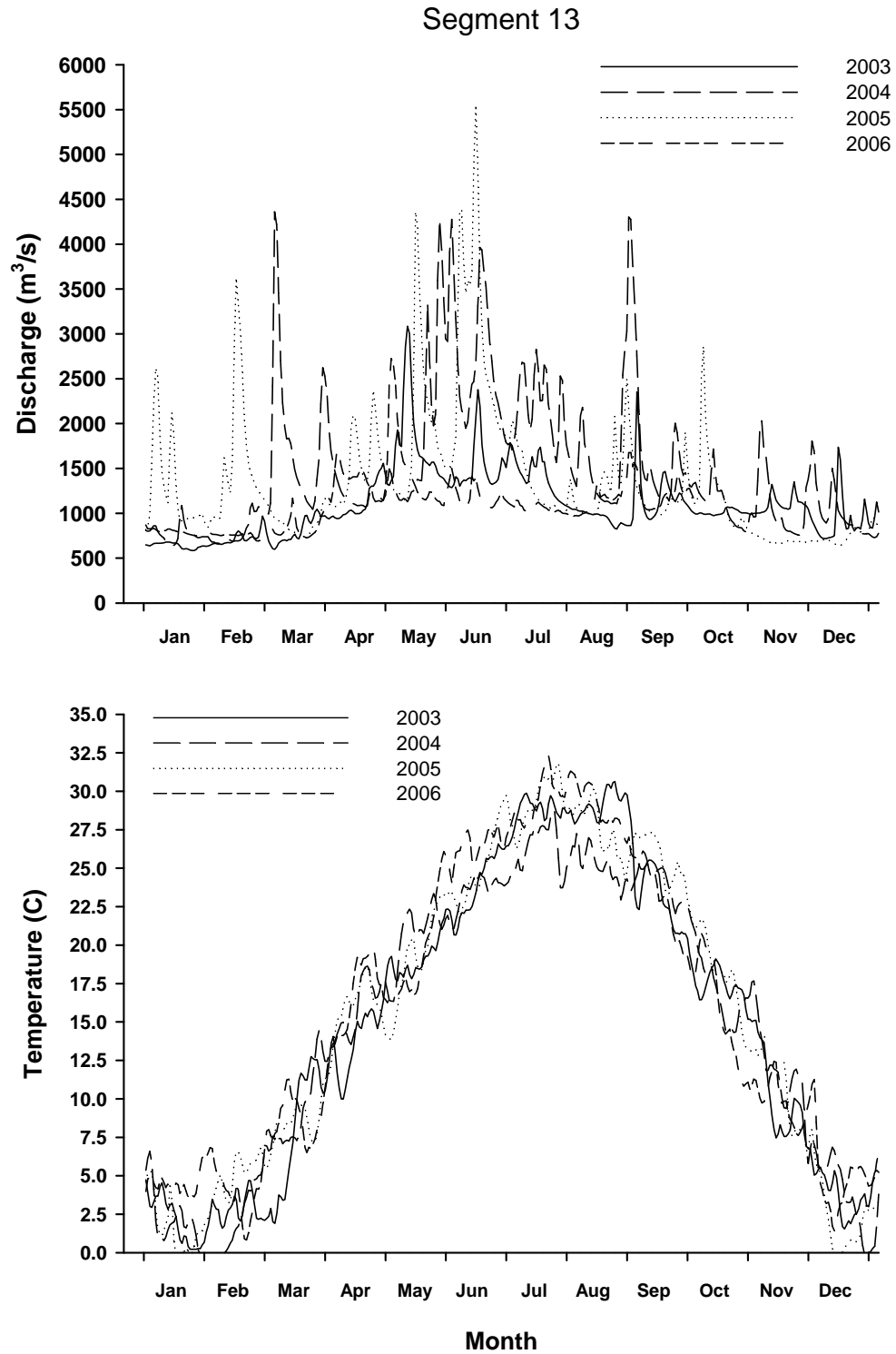


Figure 11. Mean daily discharge and mean daily water temperature for segment 13 of the Missouri River during 2003 through 2006.

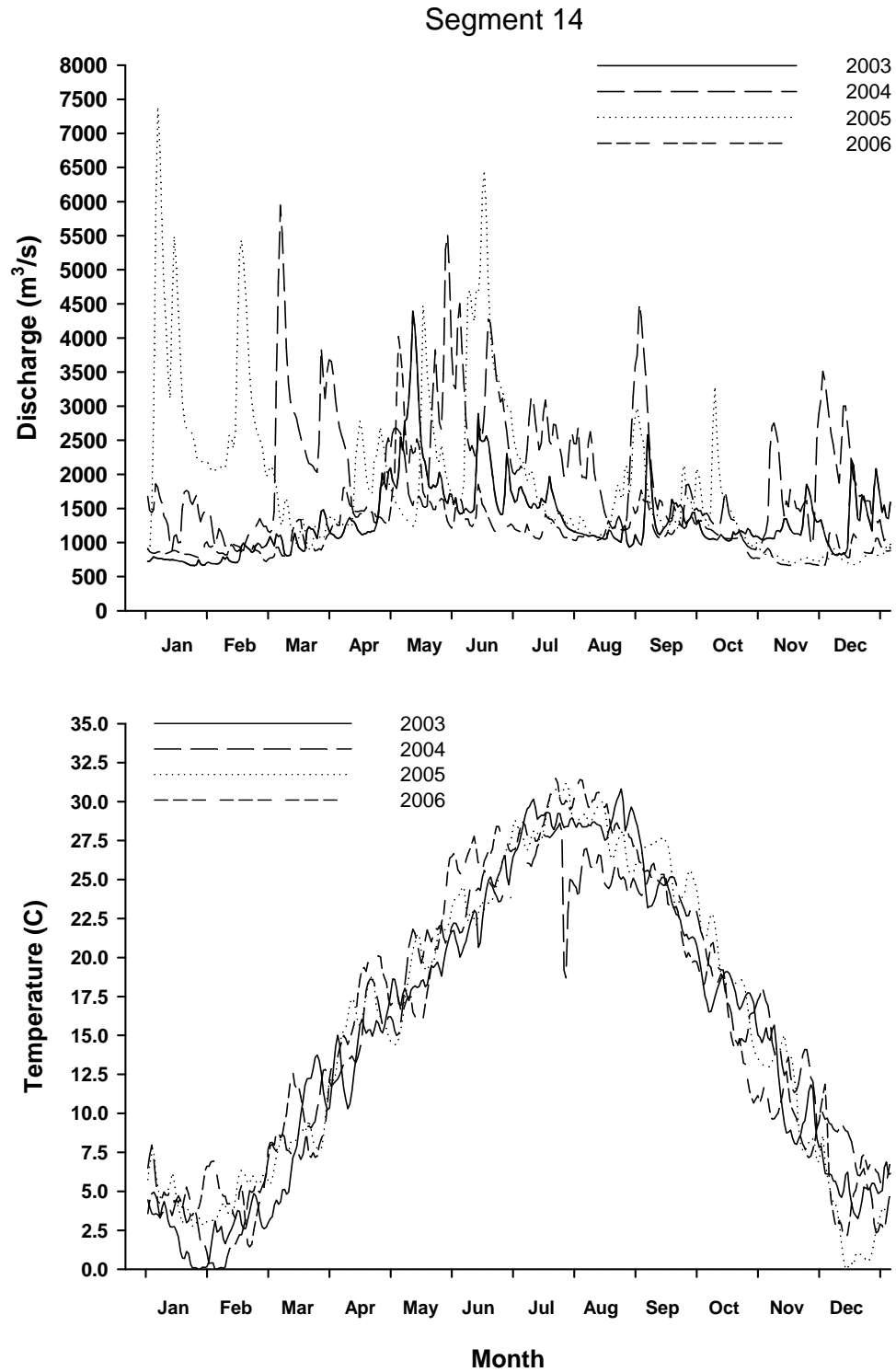


Figure 12. Mean daily discharge and mean daily water temperature for segment 14 of the Missouri River during 2003 through 2006.

Table 2. Specific dates for each year when aging structures of plains minnow/ brassy minnow/ western silvery minnow were removed.

<b>Year</b>	<b>Starting Date</b>	<b>Ending Date</b>	<b>Segments</b>
2004	August 2004	September 2004	9
2005	July 2005	October 2005	7, 8, 9, 10, 13 and 14
2006	July 2006	October 2006	2, 3, 4, 9, 13 and 14

## Results

From 2004 through 2006, 1,389 *Hybognathus spp.* were captured from all river segments (Appendix B), and aging structures were collected on 206 of these fish (Table 3). However, sample sizes were generally too small within each segment annually to statistically examine differences in growth. Mean back calculated lengths at age during 2006 were 59 mm and 84mm at age 1 and 2, respectively (Table 7; Figure 16). *Hybognathus spp.* data were tested for normality using the Kurtosis test. A parametric ANOVA with a Tukey post-hoc test showed mean length at age 0 in segment 8 & 9 in 2005 were significantly different from each other, but neither were different from the other segments where data were sufficient (Table 10). Fish collected at age 1 and older were only present in segment 7 for 2005. Fish at age 1 in Segment 9 in 2006 had the same mean length-at-capture as segments 3 and 4 in the upper universe (Table 11). All segments showed no difference of means within age 2 fish. Universe comparisons showed that fish in the upper universe were generally longer than fish in the lower universe (Table 12).

A comparison of length frequencies among segments for each year displayed no noticeable peaks in year classes. Length frequency graphs were then created monthly during fish community season for all years combined (Appendix D). The length frequency graph for July showed three age class peaks. The August-October graphs only showed two age class peaks, with the peaks shifting to the right as the year progressed.

Age frequencies were compared among segments for each year. Age frequencies for 2004 showed 100% to be age 0 (Appendix E). Age frequencies for 2005 were 98%, 1%, and 1% for age 0, 1, and 2, respectively. Age frequencies for 2006 were 42% and 44% at age 1 and 2, respectively. Fish older than age 0 in 2006 made up of 70% of the fish collected in the upper universe.

Table 3. Total number of aging structures collected for age and growth analysis.

Length	Total	2004	2005						2006					
		9	7	8	9	10	13	14	2	3	4	9	13	14
10	0													
20	7	2		2								3		
30	41	10		9	6		5	5			1	5		
40	50	10		2	6	5	4	10	2	2	2	6		1
50	34	10		1	5	3		6		6	2		1	
60	14		1	1	4		2	1	1		3	1		
70	17		1		1			1	5	3	6			
80	12		1						2	2	7			
90	17								11	4	2			
100	11								8	3				
110	3								3					
120	0													
130														



Table 4. Mean back-calculated total length-at-last annulus (+/- 2 SE) of *Hybognathus spp.* collected in each segment during 2003. A mean total length-at-age of all segments combined is also provided for each age class.

Age	Segments													Mean
	1	2	3	4	5 & 6	7	8	9	10	11	13	14		
1														
2														
3	No data for 2003													
4														
5														
6														
7														
8														
9														
10														

No data for 2003

Table 5. Mean back-calculated total length-at-last annulus (+/- 2 SE) of *Hybognathus spp.* collected in each segment during 2004. A mean total length-at-age of all segments combined is also provided for each age class.

Age	Segments													Mean
	1	2	3	4	5 & 6	7	8	9	10	11	13	14		
1														
2														
3														
4														
5														
6														
7														
8														
9														
10														

Insufficient data for 2004

Insufficient data for 2004

Table 6. Mean back-calculated total length-at-last annulus (+/- 2 SE) of *Hybognathus spp.* collected in each segment during 2005. A mean total length-at-age is not applicable because structures (> age 0) were only collected in segment 7 during 2005.

Age	Segments													Mean
	1	2	3	4	5 & 6	7	8	9	10	11	13	14		
1						53 (0..25)								
2						72 (0.00)								
3														
4														
5														
6														
7														
8														
9														
10														

Table 7. Mean back-calculated total length-at-last annulus (+/- 2 SE) of *Hybognathus spp.* collected in each segment during 2006. A mean total length-at-age of all segments combined is also provided for each age class.

Age	Segments													Mean
	1	2	3	4	5 & 6	7	8	9	10	11	13	14		
1		66 (1.00)	59 (0.86)	52 (1.38)				52 (0.00)					59 (1.43)	
2		86 (0.68)	86 (0.76)	69 (0.00)									84 (0.87)	
3														
4														
5														
6														
7														
8														
9														
10														

Figure 13. No data for 2003.

Figure 14. Insufficient data for 2004.

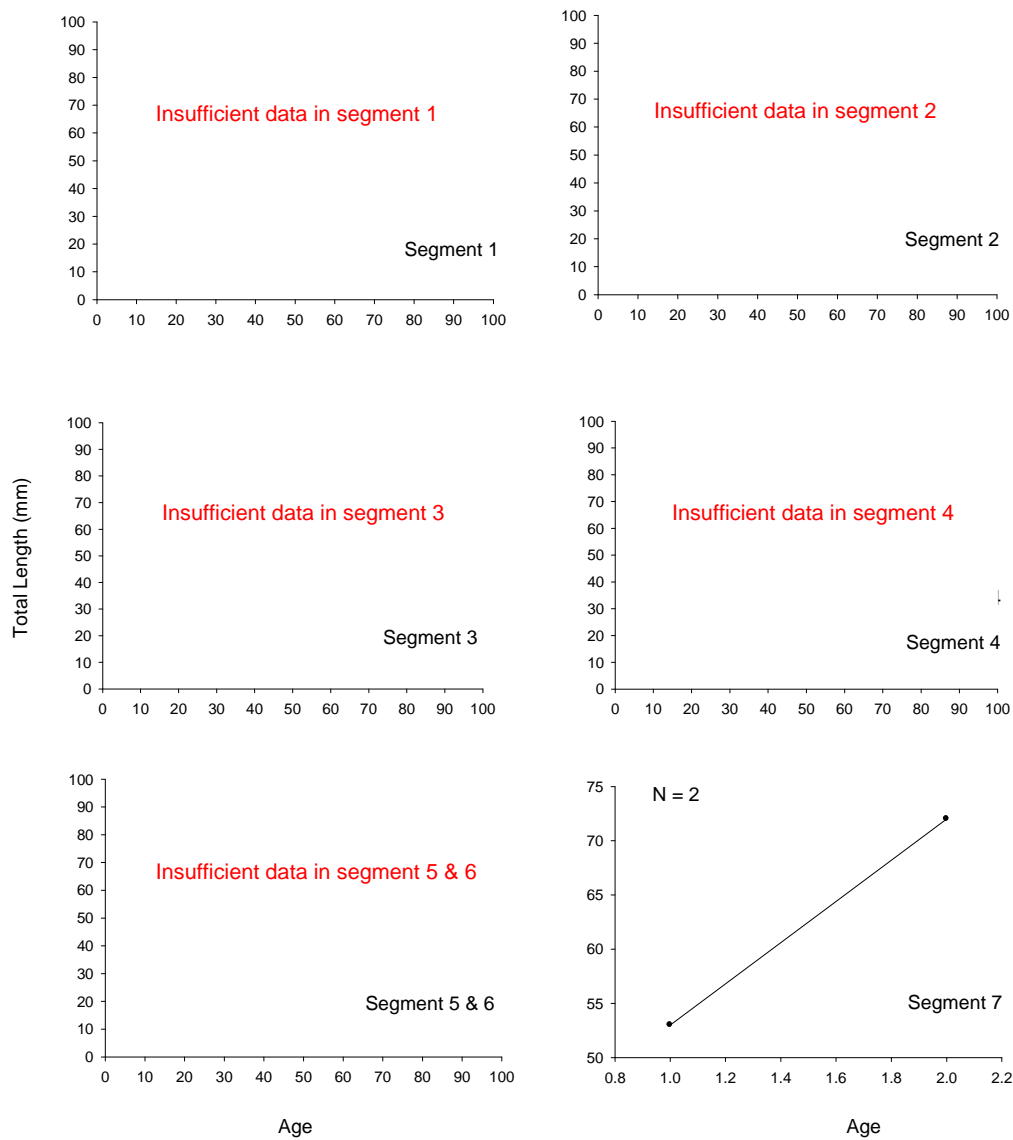


Figure 15. Mean back-calculated total length-at-last annulus curves of *Hybognathus* spp. that were collected for age and growth analysis from segments 1, 2, 3, 4, 5 & 6, 7, 8, 9, 10, 11, 13, and 14 of the Missouri River during 2005.

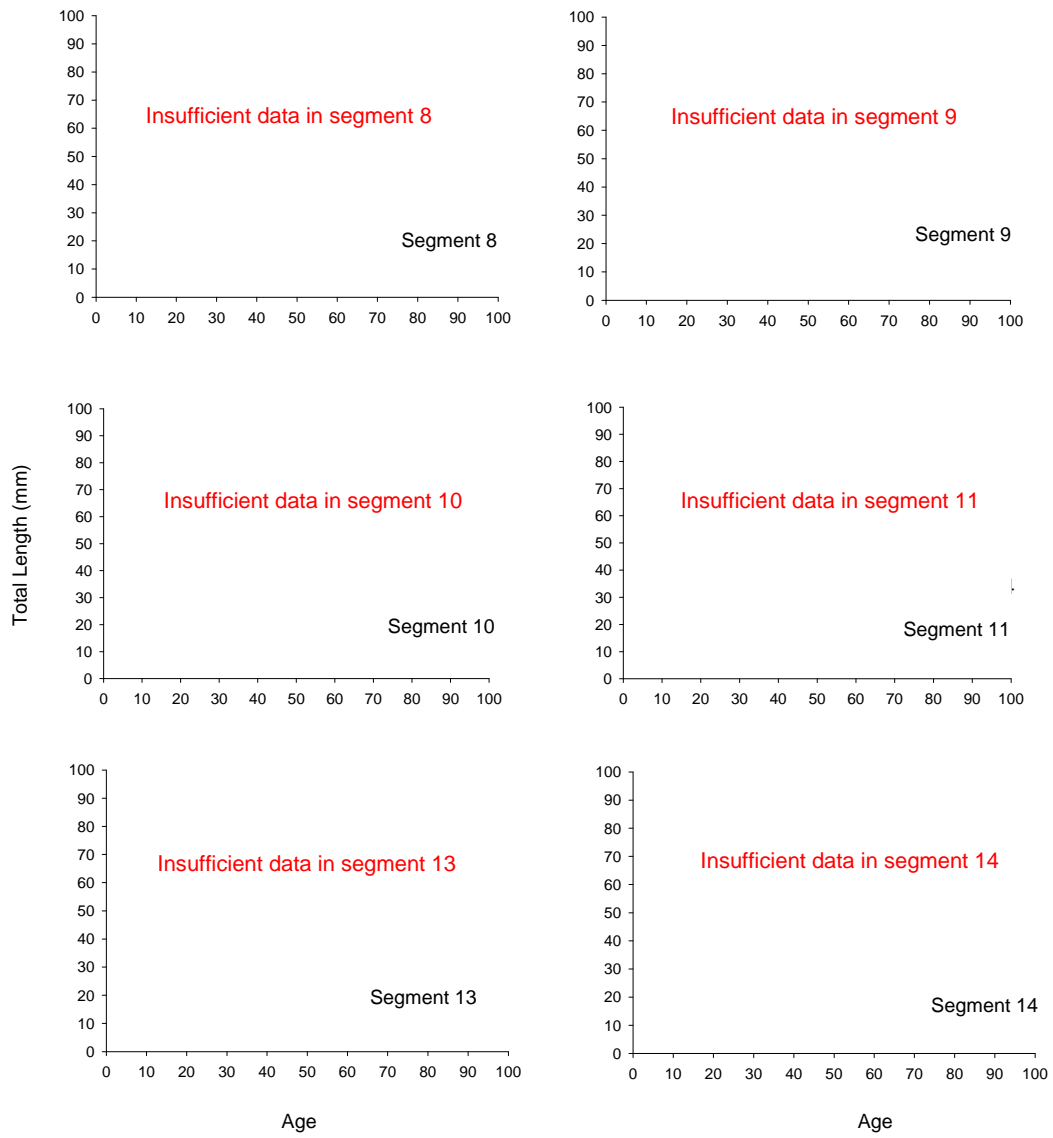


Figure 15. Continued.



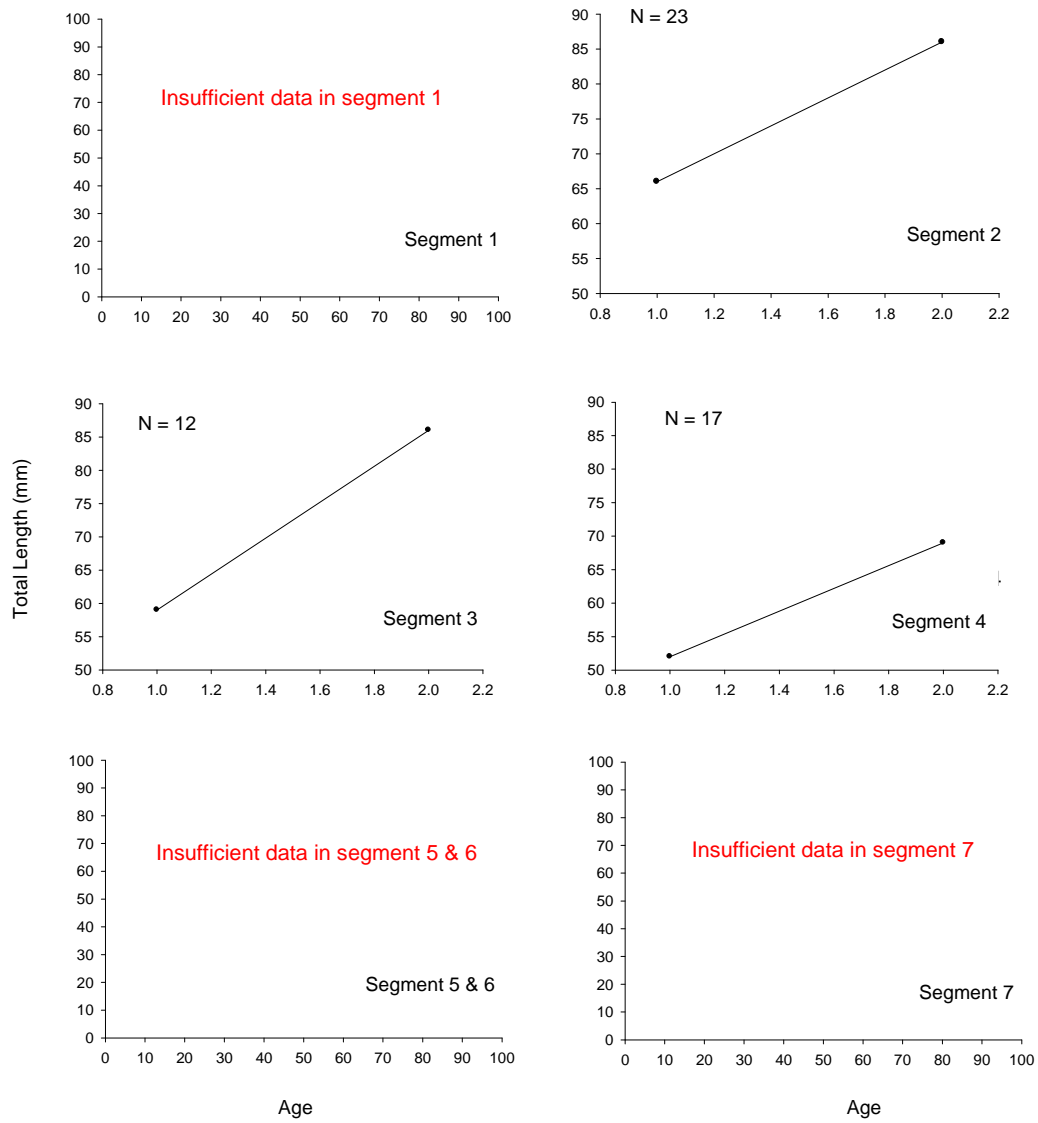


Figure 16. Mean back-calculated total length-at-last annulus curves of *Hybognathus* spp. that were collected for age and growth analysis from segments 1, 2, 3, 4, 5 & 6, 7, 8, 9, 10, 11, 13, and 14 of the Missouri River during 2006.

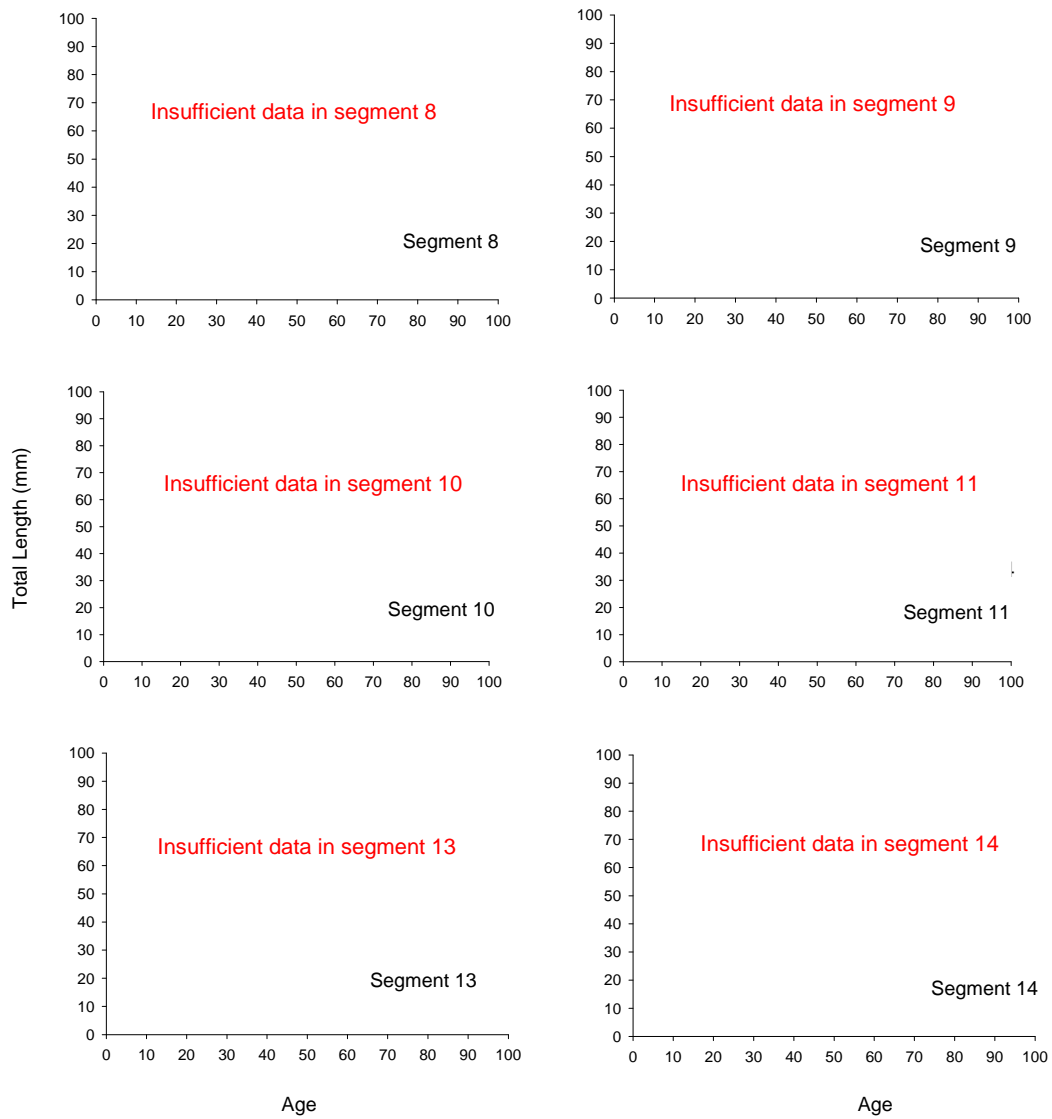


Figure 16. Continued.

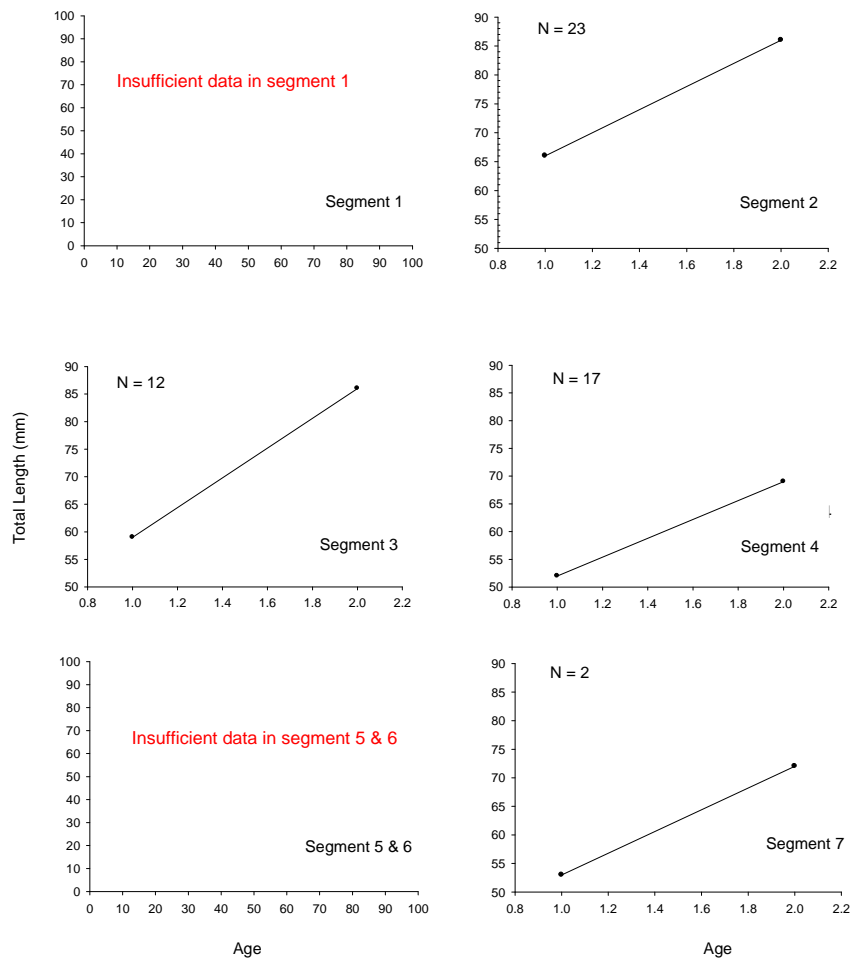


Figure 17. Mean back-calculated total length-at-last annulus curves of *Hybognathus* spp. that were collected for age and growth analysis from segments 1, 2, 3, 4, 5 & 6, 7, 8, 9, 10, 11, 13, and 14 of the Missouri River for all years combined.

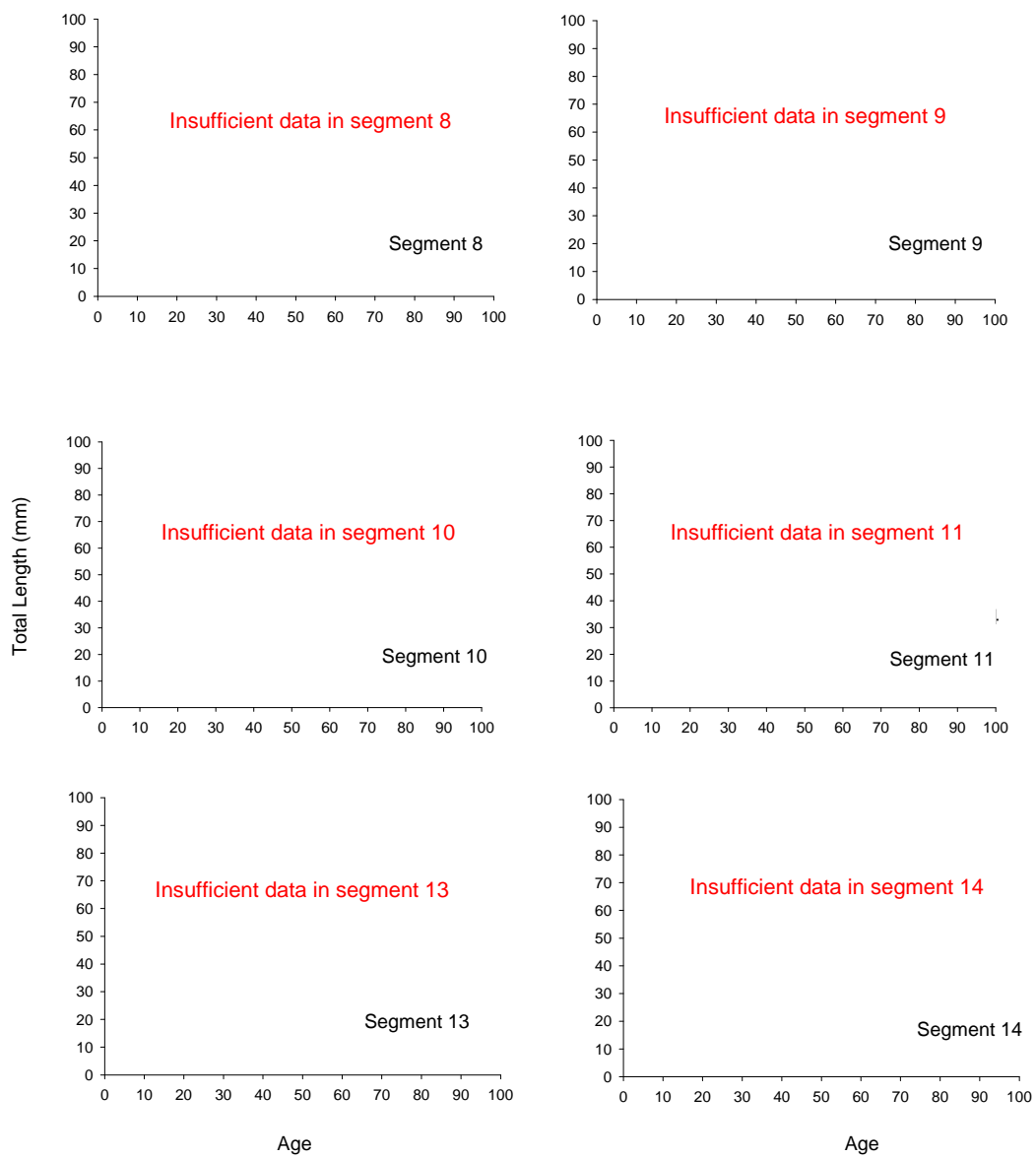


Figure 17. Continued.

Figure 18. Insufficient data to complete figure

Table 8. Mean length-at-capture comparisons of *Hybognathus spp.* between segments for 2003. Numbers below mean lengths are (+/-) 95% confidence interval and sample size, respectively. Dashes (-) indicate insufficient data to calculate confidence interval. Asterisks indicate ages tested for significant differences among segments. Segment comparisons were analyzed with a one-way ANOVA. Segments sharing a letter indicate no significant differences while different letters indicate significance differences (Tukey's test, alpha = 0.10).

Age	Segment												
	1	2	3	4	5/6	7	8	9	10	11	13	14	
0													
1													
2													
3													
4													
5													
6													
7													
8													

No data for 2003

Table 9. Mean length-at-capture comparisons of *Hybognathus spp.* between segments for 2004. Numbers below mean lengths are (+/-) 95% confidence interval and sample size, respectively. Dashes (-) indicate insufficient data to calculate confidence interval. Asterisks indicate ages tested for significant differences among segments. Segment comparisons were analyzed with a one-way ANOVA. Segments sharing a letter indicate no significant differences while different letters indicate significance differences (Tukey's test, alpha = 0.10).

Age	Segment												
	1	2	3	4	5/6	7	8	9	10	11	13	14	
0													
1													
2													
3													
4													
5													
6													
7													
8													

Insufficient data for 2004

Insufficient data for 2004

Table 10. Mean length-at-capture comparisons of *Hybognathus spp.* between segments for 2005. Numbers below mean lengths are (+/-) 95% confidence interval and sample size, respectively. Dashes (-) indicate insufficient data to calculate confidence interval. Asterisks indicate ages tested for significant differences among segments. Segment comparisons were analyzed with a one-way ANOVA. Segments sharing a letter indicate no significant differences while different letters indicate significance differences (Tukey's test, alpha = 0.10).

Age	Segment												
	1	2	3	4	5/6	7	8	9	10	11	13	14	
0*						61 ab ( -, 1)	38 a (5.1, 15)	49 b (4.6, 22)	48 ab (2.3, 8)		43 ab (6.0, 11)	47 ab (3.7, 23)	
1						87 ( -, 1)							
2						79 ( -, 1)							
3													
4													
5													
6													
7													
8													



Table 11. Mean length-at-capture comparisons of *Hybognathus spp.* between segments for 2006. Numbers below mean lengths are (+/-) 95% confidence interval and sample size, respectively. Dashes (-) indicate insufficient data to calculate confidence interval. Asterisks indicate ages tested for significant differences among segments. Segment comparisons were analyzed with a one-way ANOVA. Segments sharing a letter indicate no significant differences while different letters indicate significance differences (Tukey's test, alpha = 0.10).

Age	Segment											
	1	2	3	4	5/6	7	8	9	10	11	13	14
0*		69 a (9.0, 9)	52 b (1.8, 8)	50 b (10.4, 6)				37 c (3.4, 14)			50 abc ( -, 1)	46 abc ( -, 1)
1*		99 a (3.6, 15)	87 b (6.8, 8)	79 b (4.1, 16)				67 b ( -, 1)				
2*		101 a (5.9, 8)	100 a (7.1, 4)	82 a ( -, 1)								
3												
4												
5												
6												
7												
8												

Table 12. Mean length-at-capture comparisons of *Hybognathus spp.* between the upper and lower sampling universe. Numbers below mean lengths are (+/-) 95% confidence interval and sample size, respectively. Dashes (-) indicate insufficient data to calculate confidence interval. Asterisks indicate ages tested for significant differences among segments. Sampling universe comparisons were analyzed with a t-test. Sharing a letter indicate no significant differences while different letters indicate significance differences (alpha = 0.05).

Age	Sampling Universe	
	Upper	Lower
0*	58 a (5.6, 23)	44 b (1.7, 128)
1*	88 a (3.8, 39)	77 a ( 20.0, 2)
2	100 (5.0, 13)	79 ( -, 1)
3		
4		
5		
6		
7		
8		

Table 13. Age/length key for segment 1. Numbers in the boxes represent the probability that a known length individual is a certain age based on raw aging data from each segment.

Length Category	Age			N
	0	1	2	
20				
25				
30				
35				
40				
45				
50				
55				
60	No Data for segment 1			
65				
70				
75				
80				
85				
90				
95				
100				
105				
110				
115				

Table 14. Age/length key for segment 2. Numbers in the boxes represent the probability that a known length individual is a certain age based on raw aging data from each segment.

Length Category	Age			N
	0	1	2	
20				
25				
30				
35				
40				
45	100			2
50				
55				
60				
65	100			1
70	100			2
75	100			3
80	100			1
85			100	1
90		100		5
95		67	33	6
100		60	40	5
105		33	67	3
110		100		2
115			100	1

Table 15. Age/length key for segment 3. Numbers in the boxes represent the probability that a known length individual is a certain age based on raw aging data from each segment.

Length Category	Age			N
	0	1	2	
20				
25				
30				
35				
40				
45	100			2
50	100			4
55	100			5
60				
65				
70				
75		100		3
80				
85		100		2
90		50	50	2
95		50	50	2
100		100		1
105			100	2
110				
115				

Table 16. Age/length key for segment 4. Numbers in the boxes represent the probability that a known length individual is a certain age based on raw aging data from each segment.

Length Category	Age			N
	0	1	2	
20				
25				
30				
35	100			1
40	100			1
45	100			1
50	100			2
55				
60				
65		100		3
70	50	50		2
75		100		4
80		86	14	7
85				
90		100		1
95		100		1
100				
105				
110				
115				

Table 17. Age/length key for segments 5 and 6. Numbers in the boxes represent the probability that a known length individual is a certain age based on raw aging data from each segment.

Length Category	Age			N
	0	1	2	
20				
25				
30				
35				
40				
45				
50				
55				
60	No data for segment 5 & 6			
65				
70				
75				
80				
85				
90				
95				
100				
105				
110				
115				

Table 18. Age/length key for segment 7. Numbers in the boxes represent the probability that a known length individual is a certain age based on raw aging data from each segment.

Length Category	Age			N
	0	1	2	
20				
25				
30				
35				
40				
45				
50				
55				
60	100			1
65				
70				
75			100	1
80				
85		100		1
90				
95				
100				
105				
110				
115				



Table 19. Age/length key for segment 8. Numbers in the boxes represent the probability that a known length individual is a certain age based on raw aging data from each segment.

Length Category	Age			N
	0	1	2	
20				
25	100			2
30	100			5
35	100			4
40	100			1
45	100			1
50				
55	100			1
60	100			1
65				
70				
75				
80				
85				
90				
95				
100				
105				
110				
115				

Table 20. Age/length key for segment 9. Numbers in the boxes represent the probability that a known length individual is a certain age based on raw aging data from each segment.

Length Category	Age			N
	0	1	2	
20	100			1
25	100			4
30	100			5
35	100			16
40	100			11
45	100			11
50	100			13
55	100			2
60	100			2
65	67	33		3
70	100			1
75				
80				
85				
90				
95				
100				
105				
110				
115				

Table 21. Age/length key for segment 10. Numbers in the boxes represent the probability that a known length individual is a certain age based on raw aging data from each segment.

Length Category	Age			N
	0	1	2	
20				
25				
30				
35				
40	100			2
45	100			3
50	100			3
55				
60				
65				
70				
75				
80				
85				
90				
95				
100				
105				
110				
115				

Table 22. Age/length key for segment 11. Numbers in the boxes represent the probability that a known length individual is a certain age based on raw aging data from each segment.

Length Category	Age			N
	0	1	2	
20				
25				
30				
35				
40				
45				
50				
55				
60				
65				
70	No data for segment 11			
75				
80				
85				
90				
95				
100				
105				
110				
115				

Table 23. Age/length key for segment 13. Numbers in the boxes represent the probability that a known length individual is a certain age based on raw aging data from each segment.

Length Category	Age			N
	0	1	2	
20				
25				
30	100			3
35	100			2
40	100			2
45	100			2
50	100			1
55				
60	100			2
65				
70				
75				
80				
85				
90				
95				
100				
105				
110				
115				

Table 24. Age/length key for segment 14. Numbers in the boxes represent the probability that a known length individual is a certain age based on raw aging data from each segment.

Length Category	Age			N
	0	1	2	
20				
25				
30	100			2
35	100			3
40	100			5
45	100			6
50	100			6
55				
60	100			1
65				
70	100			1
75				
80				
85				
90				
95				
100				
105				
110				
115				

## Additional Analysis

There was not sufficient data to accurately assign an age to fish using the age/length keys based on segment (Tables 13-24). Therefore, data from each segment were combined to make an age length key for the upper (Appendix F) and lower sampling universe (Appendix G) to assign an age to all *Hybognathus spp.* sampled. Annual mortality was compared among segments for all years combined. Fish in the upper universe had an annual mortality rate of 64% while fish in the lower universe had an annual mortality rate of 98% (Appendix H).

Structure age estimations were done independently by two readers. Reader agreement was tested to determine the accuracy and precision of our results. Exact reader agreement was 87% for *Hybognathus spp.* The rate of reader agreement within +/-1 year was 100%.

## Discussion

*Hybognathus spp.* captured in the upper universe were older and larger at a given age than fish captured in the lower universe. Mortality was extremely high in the lower universe where only 3 fish were aged over 0. Scheurer et al. (2003) found that *Hybognathus spp.* survived to older ages and reached greater body lengths in upper reaches of a river system when compared to downstream portions where few survived. This species prefers sandy substrate, backwaters and pools, more of which are found in the upper universe than in the channelized lower universe. *Hybognathus spp.* also prefers tributaries of large streams (Scheurer et al. 2003). The main stem of the upper universe might have more suitable habitat for mature fish, much like the tributaries found in the lower universe.

*Hybognathus spp.* move into slow moving lower reaches of tributary streams to spawn (Miller and Robison 1973). The eggs of *Hybognathus spp.* are demersal and non-adhesive; this allows them to develop as they bounce along the river bottom. Scheurer et al. (2003) found that *Hybognathus spp.* movement rates are high, and that fish disperse over long distances to spawn. Mature *Hybognathus spp.* in the lower universe could move into the tributaries to spawn. Given the developmental characteristics of the eggs, they are likely to flush into the Missouri River during high water events. The fish may then develop in the Missouri River and, as they mature, move back into the tributaries. This hypothesis is supported by the age structure of fish in the lower universe, which show high numbers of age 0 and low numbers of mature fish.

Length frequency graphs of *Hybognathus spp.* showed three age classes in July, decreasing to two age classes by October. This could be the result of a reduction in the use of sampling gears targeting small-bodied species during this time period. Taylor and Miller (1990) used length frequency histograms to analyze age and growth data of *Hybognathus placitus* because annuli on scales could not be identified; we also found it difficult to discern annuli on scales from our collections. Scales were difficult to read because annuli were hard to distinguish. A comparison of data from our age and growth analysis to monthly length-frequency graphs showed similar age classes. Taking into account the accuracy of length frequencies to represent the age of this species and the time invested in preparation and



reading scales, we feel *Hybognathus spp.* could be accurately and more efficiently aged using length-frequency histograms.

## **Acknowledgments**

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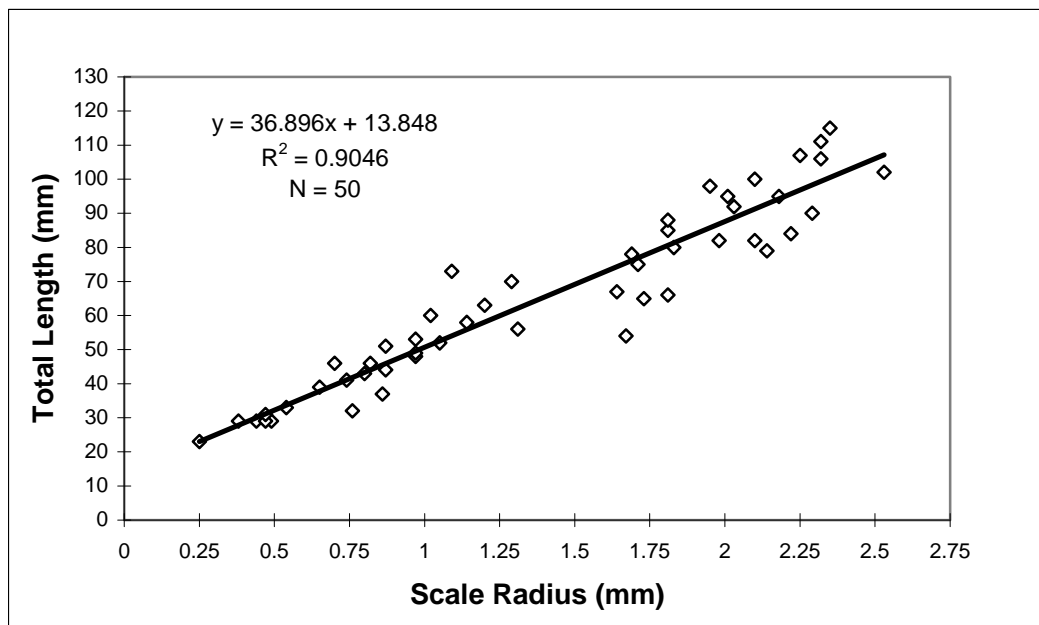
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## Appendices

Appendix A. Linear regression used to calculate Y-intercept for *Hybognathus spp.*

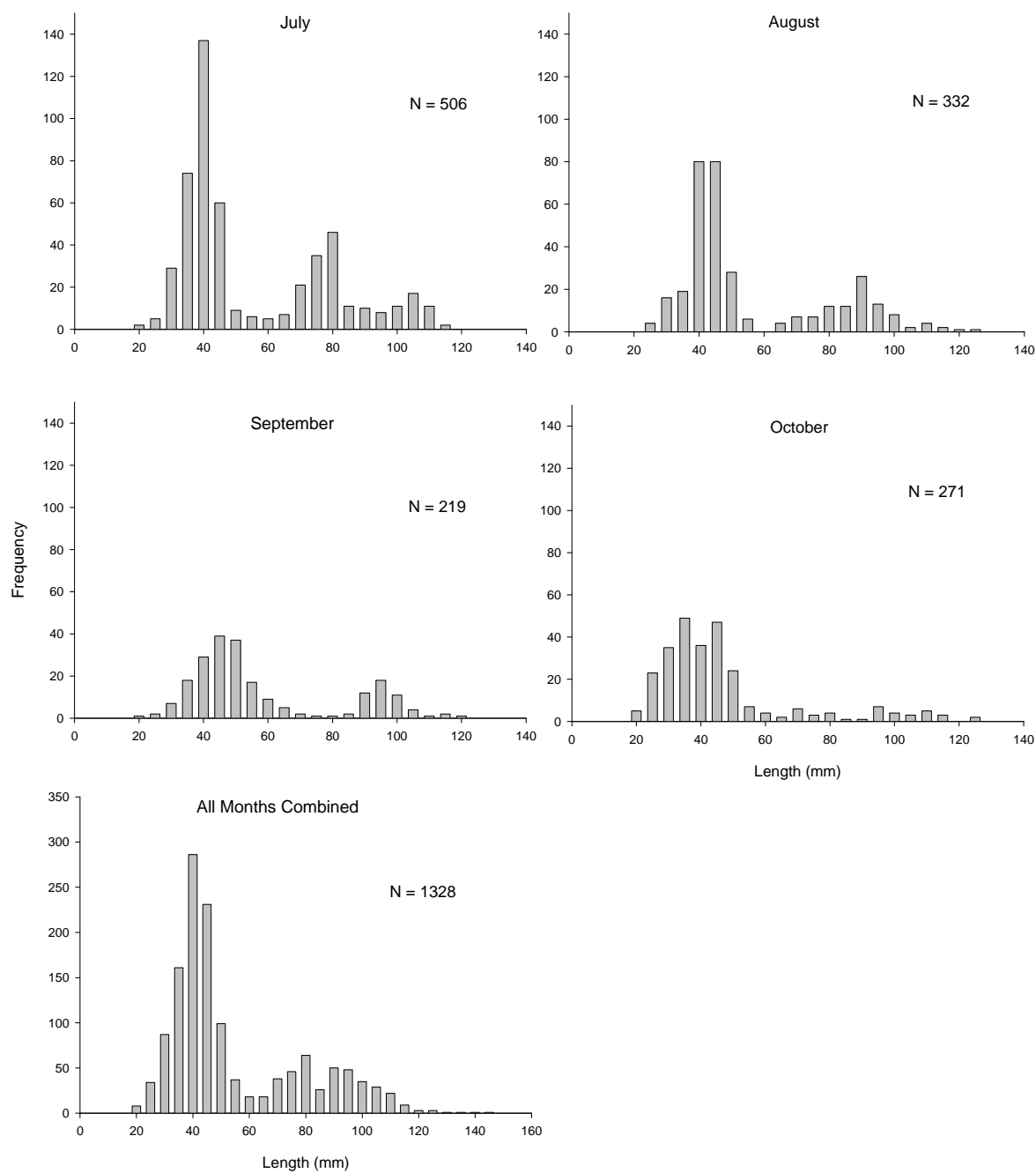


Appendix B. Total number of *Hybognathus spp.* sampled in the Missouri River for each segment during 2004, 2005, and 2006.

	2004	2005	2006	Totals
Segment 1			2	2
Segment 2			144	144
Segment 3			137	137
Segment 4		158	309	467
Segments 5 & 6		1		1
Segment 7		5	12	17
Segment 8		19	26	45
Segment 9	222	218	59	499
Segment 10		8		8
Segment 11				0
Segment 13	11	22	5	38
Segment 14	4	14	13	31
Totals	237	445	707	<b><u>1389</u></b>

Appendix C. Length-at-capture and back-calculated length comparisons between the upper and lower sampling universe for *Hybognathus spp.* for all years combined.

Age	Mean total length at capture		Mean back calculated total length	
	Upper	Lower	Upper	Lower
0	58	44	-	-
1	88	77	60	52
2	100	79	85	72
3				



Appendix D. Length frequency of all *Hybognathus* spp. collected from the Missouri River during each month of fish community season and all months combined from 2004 - 2006.



Appendix E. Age frequency tables for *Hybognathus spp.* that were collected for age and growth analysis for each segment of the Missouri River during 2004, 2005, and 2006.

2004				
Segment	Age			
	0	1	2	3
9	32			
Total	32	0	0	0
Percentage	100%	-	-	-

2005				
Segment	Age			
	0	1	2	3
7	1	1	1	
8	15			
9	22			
10	8			
13	11			
14	23			
Total	80	1	1	0
Percentage	98%	1%	1%	-

2006				
Segment	Age			
	0	1	2	3
2	9	15	8	
3	8	8	4	
4	6	16	1	
9	14	1		
13	1			
14	1			
Total	39	40	13	0
Percentage	42%	43%	15%	-

Appendix F. Age/length key for the upper universe. Numbers in the boxes represent the probability that a known length individual is a certain age based on raw aging data.

Length Category	Age			N
	0	1	2	
20				
25				
30				
35	100			1
40	100			1
45	100			5
50	100			6
55	100			5
60				
65	25	75		4
70	75	25		4
75	30	70		10
80	12.5	75	12.5	8
85		67	33	3
90		87.5	12.5	8
95		67	33	9
100		67	33	6
105		20	80	5
110		100		2
115			100	1

Appendix G. Age/length key for the lower universe. Numbers in the boxes represent the probability that a known length individual is a certain age based on raw aging data.

Length Category	Age			N
	0	1	2	
20	100			1
25	100			6
30	100			15
35	100			25
40	100			21
45	100			23
50	100			23
55	100			3
60	100			7
65	67	33		2
70	100			2
75			100	1
80				
85		100		1
90				
95				
100				
105				
110				
115				

Appendix H. *Hybognathus spp.* annual mortality rate using Heincke's method for each segment, upper universe, and lower universe of the Missouri River for all years combined.

<b>Segment Number</b>	<b>Annual Mortality</b>
3	51%
4	77%
7	40%
8	96%
9	99%
10	100%
13	100%
14	100%
Upper Universe	64%
Lower Universe	98%